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MAMMALIAN TOXICOLOGICAL EVALUATION OF P-CHLOROPHENYL METHYL SUL--ETC(U)
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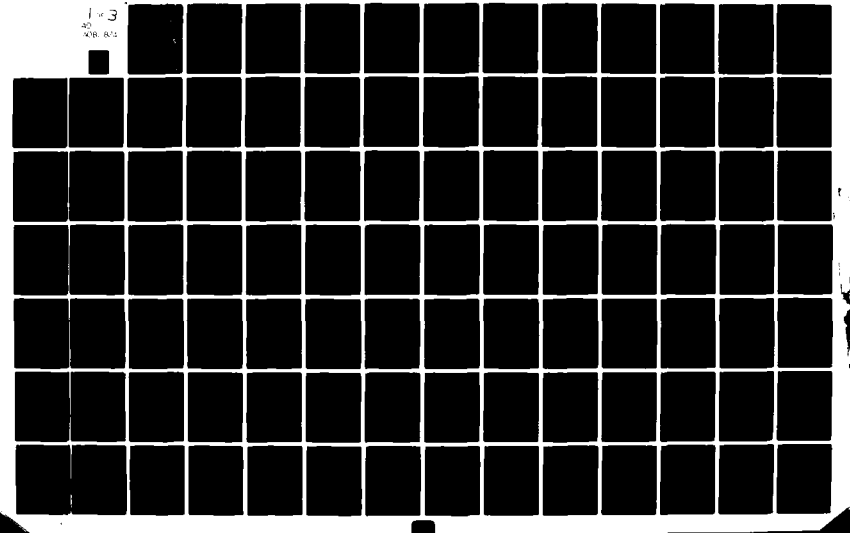

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MAMMALIAN TOXICOLOGICAL EVALUATION OF p-CHLOROPHENYL METHYL SULFIDE, p-CHLOROPHENYL METHYL SULFOXIDE, AND p-CHLOROPHENYL METHYL SULFONE

FINAL REPORT
1 Jul 77 – 31 Oct 78

Daryl C. Thake, Dennis Mays, Philip Leber, Diane Metcalf and Lalitkumar Bavda

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P-Chlorophenyl Methyl Sulfide	Fischer 344 Rats	Metaolism Studies
P-Chlorophenyl Methyl Sulfone	Rhesus Monkeys	91-Day Toxicity Studies
P-Chlorophenyl Methyl Sulfoxide	LD_{50} Studies	Reversibility Studies
$B_6C_3F_1$ Mice	Dermal Toxicity	Mutagenicity Studies
	28-Day Toxicity Studies	Pharmacokinetic Studies
		Interconversion Studies

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The purpose of this project was to determine the toxic potential of p-chlorophenyl methyl sulfide, sulfoxide and sulfone. Studies included in this report are: (1) acute oral LD_{50} in mice and rats, (2) acute dermal toxicity in rats, (3) skin irritation in rabbits, (4) eye irritation in rabbits, (5) skin sensitization in guinea pigs, (6) 28-day feeding studies in rats and mice, (7) 28-day gavage studies in rats, (8) 91-day feeding studies in rats and mice with interim sacrifices and 14-day reversibility studies, (9) 14-day gavage studies in rhesus monkeys with 14-day reversibility studies (over) -> next page

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(19 cont.)
Skin Sensitization Studies, Skin Irritation Studies, Eye Irritation Studies

(20 cont.)

(10) metabolism and pharmacokinetic studies, (11) Ames bacterial mutagenesis studies, (12) Hexobarbital sleep time studies for microsomal enzyme induction.

The LD_{50} levels for these compounds in mice and rats were as follows:

	Sulfide	Sulfoxide	Sulfone
Rats (Male	619	611	529
Rats (Female)	479	463	400
Mice (Male)	877	328	600
Mice (Female)	672	440	606

Death was achieved in the dermal toxicity studies only at the highest dosage level (5630 mg/kg). Results of the skin irritation studies in rabbits showed no irritation for sulfide, mild irritation for sulfone and sulfoxide. Sulfone induced no eye irritation in rabbits, while sulfide induced mild corneal responses which were reversible. Sulfoxide produced positive responses in cornea, iris and conjunctiva, with the corneal opacity persisting throughout the 21-day observation period. None of the 3 compounds demonstrated unequivocal skin sensitization in guinea pigs. None of the 3 compounds were mutagenic in the Ames assay. Mortality in 28-day feeding studies for rats and mice were as follows:

Rats: Sulfide 9000 ppm; sulfone >2250 ppm; sulfoxide 5200 ppm
Mice: Sulfide >4500 ppm; sulfone >4500 ppm; sulfoxide $\geq$5200 ppm

Small but statistically significant changes occurred in several hematologic and serum chemistry parameters in rats given each of the 3 compounds in the 91-day study. The most profound changes were elevated K^+ and Ca^{++} levels, decreased $N_a{}^+/K^+$ ratios and elevated BUN values, the latter only in rats treated with sulfone. Pathologic changes which were observed in this study included hepatic megalocytosis with syncitial cell formation and hepatic necrosis all of which occurred in both mice and rats given each of the 3 compounds. Flattening or denuding of bronchiolar respiratory epithelium was observed in mice given 3000 ppm of each compound.

Changes in hematologic and serum chemistry parameters in rhesus monkeys were sporadic. Compound-related pathologic changes in monkeys included hyperplastic lymphoid lesions in lymph nodes, spleen and bone marrow with one instance of a lymphoproliferative lesion in a lymph node; hepatic degeneration and necrosis, adrenal cortical hyperplasia, renal tubular vacuolization and pigmentation, thyroid follicular cell hyperplasia (mild) and vacuolization and degeneration of gastric and intestinal epithelium with evidence of cell cycle alteration.

Pharmacokinetic studies indicated biphasic disappearance of C^{14} labelled compounds with greatest urinary excretion in rats on day 2 and in monkeys on day 7. Fecal elimination was minor for all compounds. Metabolism studies indicated that rats converted all 3 chemicals to water soluble compounds to a greater extent than did monkeys. Analytical studies revealed extensive interconversion of sulfide and sulfoxide to sulfone. Hexobarbital sleep time studies clearly demonstrated that all 3 compounds are potent microsomal enzyme inducers.

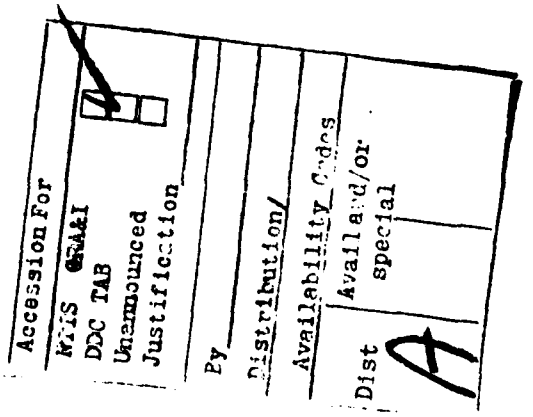
SUMMARY

The purpose of the studies described in this report was to determine the toxic potential of p-chlorophenyl methyl sulfide, p-chlorophenyl methyl sulfoxide, and p-chlorophenyl methyl sulfone. This included (1) identification of target organs, (2) dose response data, (3) evaluation of reversibility of toxic changes, (4) evaluation of metabolic and pharmacokinetic parameters to include in vivo interconversion studies, and (5) determination of mutagenicity.

A number of different studies were included in the work performed on this project and described in this report. These include:

(1) Acute oral toxicity (LD_{50}) studies in rats and mice
(2) Acute dermal toxicity studies in rats
(3) Skin irritation studies in rabbits
(4) Eye irritation studies in rabbits
(5) Skin sensitization studies in guinea pigs
(6) 28-day feeding studies in rats and mice
(7) 28-day oral gavage studies in rats
(8) 91-day feeding studies in rats and mice with interim sacrifices and 14-day reversibility studies
(9) 14-day oral gavage studies in rhesus monkeys with 14-day reversibility studies
(10) Metabolism and pharmacokinetic studies
(11) Ames bacterial mutagenesis assays
(12) Hexobarbital sleep time studies for microsomal enzyme induction.

The acute oral toxicity studies were conducted using Fischer 344 rats and $B_6C_3F_1$ mice. At least four dosage levels were established for each sex between 0 and 100 % lethality. Results of the probit analyses are as follows (results expressed as mg/kg):



Rats

	Sulfide		Sulfone		Sulfoxide	
	Male	Female	Male	Female	Male	Female
LD_{10}	534	326	459	324	483	398
LD_{50}	619	479	529	400	611	463
LD_{90}	719	703	608	494	773	539

Mice

	Sulfide		Sulfone		Sulfoxide	
	Male	Female	Male	Female	Male	Female
LD_{10}	681	664	563	572	227	341
LD_{50}	877	672	600	606	328	440
LD_{90}	1130	744	639	641	473	566

Male rats were generally less sensitive than were females to all three compounds. The same was true for the LD_{50} and LD_{90} levels in mice given sulfide; however, female mice were less sensitive to sulfoxide than were males and both sexes were equally as sensitive to sulfone. Sensitivity of mice as compared to rats varied with each compound as is seen in the table shown above. Sulfone was slightly more toxic than sulfide or sulfoxide in rats while sulfoxide was substantially more toxic than the other compounds in mice.

Acute dermal toxicity studies were conducted for all three compounds in Fischer 344 rats. In order to apply enough solution to obtain lethality, the compounds were applied to gauze sponges which were secured to shaved areas of skin by adhesive bandages. A single 24-hour exposure with a 14-day observation period was used. Two of two males and two of two females treated with the highest dose of sulfide (5630 mg/kg) died. No deaths occurred in the other two compounds at any dosage level and no deaths occurred in rats treated with lower doses of sulfide.

The skin irritation studies were conducted using young New Zealand White rabbits. The three compounds, each of which was dissolved in corn oil, were applied to the skin by securing surgical dressings containing measured amounts of the compounds to the shaved test sites on

the animals. Exposure lasted 24 hours. Test sites were evaluated at 24, 48, and 72 hours posttreatment and at longer periods if the skin had not returned to normal. Grading of lesions was according to the Draize procedure. Sulfide produced no irritation and sulfone produced mild irritation in one male and one female. Sulfoxide produced mild irritation in two males and two females and severe irritation in one female.

Eye irritation studies were conducted in New Zealand White rabbits using the modified Draize procedure. Measured amounts of the undiluted compounds were applied to the conjunctival sac and eyes were evaluated at 2, 24, 48, and 72 hours at 7, 14, and 21 days (the last two time periods only if lesions were present at 7 days). Sulfone did not induce irritation while sulfide induced mild corneal responses in four rabbits which were reversible by 7 days posttreatment. Sulfoxide produced positive responses in the cornea, iris, and conjunctiva in all rabbits tested. Responses in the iris and conjunctiva were reversible by 7 days while corneal opacity persisted throughout the 21-day observation period.

Skin sensitization studies were conducted in female Hartley strain guinea pigs. The procedure consisted of an induction phase in which Freund's complete adjuvant only, test compound, and test compound plus Freund's complete adjuvant were injected at three sites in two parallel rows. The test agent was applied topically to the injection sites 1 week after the injections. The challenge was accomplished by topical application of the test agent 14 days following the initial topical application. The challenge site was evaluated 24 and 48 hours after the challenge. Specimens were collected for histologic examination. Extremely mild changes were observed microscopically in three animals from both the sulfone and sulfoxide groups; no changes were observed in the sulfide group. The nature and severity of these changes were such that the lesions were considered equivocal as evidence of sensitization.

Ames bacterial mutagenesis assays were performed using each of the test compounds. These tests were conducted with and without microsomal activation, the microsomal induction for which was accomplished by using both Arochlor 1254 and phenobarbital in separate assays. None of the test compounds were mutagenic in this system with either Arochlor- or phenobarbital-induced microsomes or in tests where microsomal activation was not used.

Twenty-eight day range finding studies were conducted using all three compounds in both $B_6C_3F_1$ mice and Fischer 344 rats. The purpose of these studies was to determine appropriate dosage levels for the 91-day studies. Since palatability was a problem at high dosage levels when these compounds were incorporated into the diets of rats, parallel 28-day gavage studies were conducted along with dose-feed studies in rats, while just dosed feed studies were conducted in mice. Mortality data as well as body weight and food consumption data were used to determine dosage levels for the 91-day feeding studies. Mortality data from the 28-day range finding studies are shown below. Dosage levels shown are the lowest at which mortality occurred. Numbers in parentheses represent the incidence of mortality.

	Sulfide		Sulfone		Sulfoxide	
	Male	Female	Male	Female	Male	Female
Dosed feed rats, ppm	9000(5/5)	9000(5/5)	>2250	>2250	5200(4/5)	5200(3/5)
Dosed feed mice, ppm	>4500	>4500	>4500	>4500	>5200	5200(2/5)
Gavage rats, mg/kg	150(1/5)	37.5(2/5)	50(3/5)	50(3/5)	400(5/5)	200(1/5)

Both body weights and food consumption were substantially decreased, especially at the higher dosage levels in both oral and gavage studies in rats. Body weight and food consumption data for mice were extremely erratic and trends were difficult to verify due to these inconsistencies.

Ninety-one day feeding studies were conducted using $B_6C_3F_1$ mice and Fischer 344 rats. Interim sacrifices occurred at 28 and 63 days with 14-day recovery groups at all dosage levels for all sacrifice intervals in both mice and rats. Clinical chemistry and hematology parameters were evaluated in rats terminated after 91 days of exposure to the test compounds, including both the groups that were terminated before and after the 14-day recovery period.

Body weight and food consumption data showed similar relationships for all three compounds. Food consumption was decreased early with recovery

during the later weeks in the study; body weight was comparably decreased early but did not recover and remained depressed throughout the study.

Mild but statistically significant decreases in SGOT and alkaline phosphatase levels were observed in male rats given sulfone and sulfide at all levels. These remained low following the 14-day recovery period although the difference from control levels of SGOT was not statistically significant. Blood urea nitrogen values were significantly elevated in male rats treated with sulfone at all levels. Small but statistically significant changes were observed in many hematologic parameters for both males and females treated with all three compounds. Decreases in hemoglobin, hematocrit, and red blood cell levels were considered to be biologically significant. Serum potassium and calcium levels were elevated in rats given all three compounds, and sodium/potassium ratios were substantially decreased. Pathologic changes which were observed in this study included hepatic megalocytosis with syncitium formation and hepatic necrosis, all of which were observed in both mice and rats from all three studies. Necrosis occurred in only one animal following the recovery period and the incidence and severity of other hepatic lesions was decreased following recovery. Flattening and/or denudation of bronchiolar respiratory epithelium was observed in mice given 3000 ppm of all three compounds. Organ weights were generally depressed in accordance with the decreased body weights as compared to controls. Liver weights were markedly increased which correlated with the microscopic changes. Kidney weights were increased in all three compounds, being most profound at the 1500 and 750 ppm levels. Seminal vesicle and uterus weights were markedly depressed in rats which received all three compounds. Electrocardiograms in rats treated with all three compounds revealed no relevant changes from control animals.

Male and female rhesus monkeys were used for a 14-day oral gavage study using all three compounds. One-half of the monkeys were terminated at the end of the 14-day treatment period and one-half were terminated following a 14-day recovery period. Clinical chemistry and hematology parameters were evaluated; ophthalmic and EKG examinations were performed. Dose response data were similar for all three compounds. Death occurred in animals given 20 mg/kg of sulfide and sulfone while severe depression

occurred in animals given 20 mg/kg of sulfoxide. Minimal effects were observed at the lowest levels, 2.5 or 5.0 mg/kg, with the exception of a lymphoproliferative lesion which occurred in one animal given 5 mg/kg of sulfoxide. Depression, anorrhexia, diarrhea, and emesis were the most prominent symptoms induced by all compounds. Decreased hemoglobin, hematocrit, and erythrocyte levels were observed in several animals at the lethal dose level of sulfoxide; otherwise, changes in hematologic parameters were sporadic. Blood urea nitrogen and SGOT values were increased at higher dosage levels in animals from all three studies. Alkaline phosphatase levels were depressed in several animals given 10 mg/kg or 20 mg/kg of sulfide with no evidence of recovery. Other changes in clinical chemistry parameters were sporadic and inconsistent. No relevant ophthalmic or EKG changes were observed in this study. Compound-related pathologic changes were observed in several organs and were generally consistent for animals treated with all three compounds. These included lymphoreticular proliferative lesions in lymph nodes, spleen, and bone marrow; lymphoid depletion; hepatocellular vacuolization, degeneration, and necrosis; vacuolization and pigmentation of renal tubular epithelium; adrenal cortical hyperplasia; thyroid follicular cell hyperplasia; and vacuolization and degeneration of gastric and intestinal epithelium with cell cycle alterations in a few animals. These lesions were generally limited to or were more severe in animals from the higher dosage groups with the exception of the lymphoreticular proliferative lesions which also occurred in lower dosage groups. These lesions occurred for the most part in animals terminated immediately following the treatment period, although occasional lesions were observed in monkeys following the recovery period.

Metabolism and pharmacokinetic studies were conducted in rats and monkeys. ^{14}C-labeled compounds were administered to rats and monkeys. Blood, urine, and fecal samples were collected at predetermined intervals and levels of the radiolabeled compounds were measured at each interval. The urinary concentrations of each compound were determined prior to and following acid hydrolysis. *In vivo* interconversion of sulfide, sulfoxide, and sulfone in rats was determined by gas chromatographic mass spectrophotometric analysis. Results of pharmacokinetic studies indicated

a biphasic disappearance of ^{14}C in rats for all three compounds. Urinary excretion of these compounds in rats was greatest the first day while that of monkeys was constant for 7 days. Renal elimination in monkeys was saturable while that of rats was not. Fecal elimination was minor for all compounds in both species. Results of metabolism studies indicated that rats converted the chemicals to water-soluble compounds to a greater extent than did monkeys. Both species conjugate these compounds except for sulfoxide in which only limited conjugation occurred in rhesus monkeys. Analytical studies revealed extensive in vivo interconversion of sulfide and sulfoxide to sulfone.

Hexobarbital sleep time studies were performed in male rats as a measure of microsomal enzyme induction. Results clearly demonstrate that sulfide, sulfoxide, and sulfone are potent inducers of microsomal enzymes.

CONCLUSIONS

- There was no evidence of mutagenicity for any of the three compounds in this study in the Ames bacterial mutagenesis assay.
- There was substantial in vivo conversion of sulfoxide and sulfide to sulfone in rats in this study.
- All three compounds are potent microsomal enzyme inducers as determined by hexobarbital sleep time studies.
- Major morphologic lesions in rodents occurred in the liver and lung.
- Major lesions in monkeys occurred in the lymphoid system, liver, gastrointestinal tract, kidney, and adrenal glands.
- All three compounds are capable of inducing lymphoproliferative lesions in rhesus monkeys after short exposure periods and at low dosage levels.
- The "no effect" level for all three compounds in $B_6C_3F_1$ mice and Fischer 344 rats is less than 750 ppm for 91-day studies.
- The "no effect" level for these compounds in rhesus monkeys is below 5 mg/kg/day for 14-day studies.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Copies of the general methods and raw data are available at the Environmental Protection Research Division, US Army Medical Bioengineering Research Laboratory, Fort Detrick, Frederick, Maryland.

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MAMMALIAN TOXICOLOGICAL EVALUATION OF p-CHLOROPHENYL METHYL SULFIDE, p-CHLOROPHENYL METHYL SULFOXIDE, AND p-CHLOROPHENYL METHYL SULFONE

INTRODUCTION

An important series of selective herbicides was introduced into agriculture in the 1960s. The dinitroanilines, long recognized as dye intermediates, were shown to have fungicidal activity. In 1966, Planavin (nitralin; 4-[methylsulfonyl]-2,6-dinitro-N,N-dipropyaniline) was introduced and quickly gained widespread usage for the selective pre-emergence control of a wide variety of grasses and broadleaf weeds. The acceptance of and demonstrated need for Planavin and other effective, dependable dinitroaniline-derived herbicides resulted in increased production of the herbicides. With increased production volume, sizeable amounts of unreacted intermediates, degradation products, and/or impurities were formed. These chemicals have been identified in the ground water; therefore, it is necessary to determine the toxicity of these substances in mammalian systems.

The three chemicals in this report include p-chlorophenyl methyl sulfide, p-chlorophenyl methyl sulfoxide, and p-chlorophenyl methyl sulfone. These chemicals are intermediates in the manufacture of Planavin. Prior to these studies, very little information was available regarding the toxicity of the intermediates to mammalian systems. The studies described in this report define the acute and subchronic toxicity in rodents and rhesus monkeys, the metabolism, disposition and pharmacokinetics, in vivo mutagenicity, enzyme induction, irritation, and sensitivity results.

PURITY OF TEST CHEMICALS

The unlabeled sulfide, sulfoxide, and sulfone were synthesized by Parish Chemical Company. The corresponding ring-^{14}C-labeled compounds were synthesized by the New England Nuclear Company.

Using the gas chromatographic procedure described in Appendix D of the Technical Report, the sponsor determined the purities of the unlabeled sulfide and sulfone were 95% and 99%, respectively. In each case the impurities were the other two test materials.

The purity of the unlabeled sulfoxide was determined at Battelle using a gas chromatographic procedure. The compound was dissolved in chloroform and injected into a Model 2100 Varian Aerographic gas chromatograph equipped with a flame ionization detector and a 6' x 1/8" I.D. glass column packed with 10% FFAP on 80-100 mesh Chromosorb W-AW. Column temperature was programmed from 140 to 240°C at 10°C/minute. The injection port temperature was 240°C and the detector temperature was 250°C. Under these conditions, retention times for the sulfide, sulfoxide, and sulfone were 5.0, 11.5, and 14.2 minutes, respectively. Using the above method, the unlabeled sulfoxide was found to contain 95.9% sulfoxide and 4.1% sulfone.

The purities of the labeled compounds were determined by the New England Nuclear Company using either thin layer chromatography or gas liquid chromatography. The radiochemical purity for all three compounds was greater than 99%.

ACUTE ORAL TOXICITY STUDIES IN RATS AND MICE

METHODS

The oral toxicities of p-chlorophenyl methyl sulfide, sulfone, and sulfoxide following a single dose were determined in Fischer 344 rats and $B_6C_3F_1$mice. Range finding studies involving three animals per sex per dose were performed by administering doses in increasing logrithmic intervals until a definite point of toxicity was established. Based on the results of the range finding studies, groups of 10 animals per sex per dose were treated to determine the LD_{50} and the slopes of the dose response curves. Data were recorded separately for each sex.

A least four dosage levels were established for each sex which covered the range from 0 to 100% lethality. At least one dose level was between 0% and 10% lethality, at least two dose levels were between 10% and 90% lethality, and at least one dose level was between 90% and 100% lethality.

All drug effects, recovery rates, days of death, and other pertinent toxicologic information were recorded.

Young adult animals were used in all studies. The animals, supplied by Charles River Laboratories, were housed in polycarbonate cages (size 9-1/2 in. long x 10 in. wide x 6 in. high) with stainless steel barred lids, three or four rats per cage, or 10 mice per cage. Water was supplied via glass bottles with rubber stoppers placed on the top of each cage. Pelleted feed was supplied ad libitum (Wayne Laboratory Animal Diets, Lab-Blox F-6, Allied Mills, Inc., Chicago, Illinois). Constant temperature and humidity and light/dark cycle of 12 hours was maintained. The animals were placed under quarantine for a period of at least 1 week and were observed daily for any abnormalities.

After being released from quarantine, the animals were housed under the conditions stated above. Each rat was given a temporary ideni-fication number and was weighed using a Mettler PT15 scale. All rodents were randomized according to the technique described by Goldstein (1964). Animals were permanently identified by toe punching.

The compounds were dissolved in corn oil and administered by oral gavage. Dosage levels were varied by varying compound concentration while keeping volume constant on a milligram per kilogram body weight basis. A 3-inch, 16-gauge stainless steel feeding tube and a 1.5 inch, 18-gauge stainless steel feeding tube (Popper and Sons, Inc., New Hyde Park, New York) were used for rats and mice, respectively. Animals were observed twice daily for mortality and pharmocotoxic symptoms.

Log-probit analyses were performed separately for each species and sex. Data were combined for sexes where appropriate.

RESULTS

The results of the acute oral toxicity study in rats and mice are presented in Tables 1-6.

The sulfide and sulfoxide were readily soluble in the corn oil vehicle while sulfone was soluble up to a concentration of approximately 20 mg/ml. This limited solubility of sulfone resulted in the administration of suspensions at dosage levels greater than 562 mg/kg. Special precautions were taken to insure that the mixtures remained homogeneous during compound administration.

Time of death ranged from 4 hours at the highest dose to 7 days at the lowest lethal dose. Pharmacotoxic symptoms observed with both compounds included an immediate decrease in locomotor activity at all doses tested. At higher doses this was followed by loss of coordination, prostration, loss of consciousness, labored respiration, and death. Severe lacrimation was characteristic of the animals treated with sulfide and sulfoxide. Diarrhea was observed in animals treated with sulfone at the higher levels. The cause of death appeared to be complicated by the secondary effects, dehydration and starvation, observed in animals that were prostrate for extended periods of time.

Male rats were consistently more resistant than female rats to the compounds as evidenced by their higher LD_{50} values. Sulfide and sulfoxide were equally toxic to rats while sulfone was slightly more toxic to both male and female rats. This difference could be due in

TABLE 1. ACUTE ORAL TOXICITY OF p-CHLOROPHENYL METHYL SULFONE IN RATS

Male		Female	
Dose, mg/kg	Mortality	Dose, mg/kg	Mortality
708	9/10	473	10/10
562	10/10	447	11/20
531	6/10	417	11/20
501	9/10	398	15/20
484	1/20	355	3/20
464	0/10	316	1/10
Control	0/10	282	0/10
		Control	0/10

PROBIT ANALYSIS RESULTS[(a)]

	Male	Female
LD_{10}	459(380-556)	324(279-376)
LD_{50}	529(467-598)	400(372-430)
LD_{90}	608(445-826)	494(426-574)

[(a)] Doses with 95% confidence intervals are expressed in mg/kg.

TABLE 2. ACUTE ORAL TOXICITY OF p-CHLOROPHENYL METHYL SULFIDE IN RATS

Male		Female	
Dose, mg/kg	Mortality	Dose, mg/kg	Mortality
708	10/10	794	18/20
681	8/10	708	10/10
656	8/10	631	14/20
		562	10/10
631	2/10	501	7/10
501	1/10	398	3/10
398	0/10	355	0/10
Control	0/10	Control	0/10

PROBIT ANALYSIS RESULTS[a]

	Male	Female	Male and Female
LD_{10}	534(473-603)	326(235-453)	380(293-493)
LD_{50}	619(556-655)	479(407-563)	535(471-606)
LD_{90}	719(666-775)	703(571-866)	751(674-905)

[a]Doses with 95% confidence intervals are expressed in mg/kg.

TABLE 3. ACUTE ORAL TOXICITY OF p-CHLOROPHENYL METHYL SULFOXIDE IN RATS

Male		Female	
Dose, mg/kg	Mortality	Dose, mg/kg	Mortality
794	9/10	631	10/10
708	12/20	562	9/10
631	12/20	501	8/10
562	5/10	447	4/10
501	1/10	398	1/10
447	0/10	355	0/10
398	0/10	316	0/10
Control	0/10	Control	0/10

PROBIT ANALYSIS RESULTS[(a)]

	Male	Female	Male and Female
LD_{10}	483(433-539)	398(366-433)	399(339-470)
LD_{50}	611(574-652)	463(439-489)	543(495-597)
LD_{90}	773(688-869)	539(496-587)	740(618-886)

[(a)]Doses with 95% confidence intervals are expressed in mg/kg.

TABLE 4. ACUTE ORAL TOXICITY OF p-CHLOROPHENYL METHYL SULFONE IN MICE

Male		Female	
Dose, mg/kg	Mortality	Dose, mg/kg	Mortality
794	10/10	794	10/10
631	9/10	631	8/9
613	5/10	613	5/10
596	5/10	596	3/10
579	4/10	579	3/10
562	0/10	562	0/10
Control	0/10	Control	0/10

PROBIT ANALYSIS RESULTS[(a)]

	Male	Female	Male and Female
LD_{10}	563(543-585)	572(554-590)	568(554-581)
LD_{50}	600(588-612)	606(594-617)	603(595-611)
LD_{90}	639(621-690)	641(616-667)	640(622-659)

[(a)]Doses with 95% confidence intervals are expressed in mg/kg.

TABLE 5. ACUTE ORAL TOXICITY OF p-CHLOROPHENYL METHYL SULFIDE IN MICE

Male		Female	
Dose, mg/kg	Mortality	Dose, mg/kg	Mortality
1122	10/10	1000	9/10
1000	13/20	891	8/10
944	8/10	857	10/10
891	5/10	825	10/10
841	3/10	708	2/10
631	1/10	631	0/10
501	0/10	Control	0/10
Control	0/10		

PROBIT ANALYSIS RESULTS[a]

	Male	Female	Male and Female
LD_{10}	681(580-799)	664(664-811)	635(536-752)
LD_{50}	877(817-941)	672(672-858)	804(738-876)
LD_{90}	1130(1003-1272)	744(744-1012)	1019(893-1164)

[a]Doses with 95% confidence intervals are expressed in mg/kg.

TABLE 6. ACUTE ORAL TOXICITY OF p-CHLOROPHENYL METHYL SULFOXIDE IN MICE

Male		Female	
Dose, mg/kg	Mortality	Dose, mg/kg	Mortality
501	9/10	631	10/10
447	9/10	562	9/10
398	6/10	501	4/10
355	7/10	447	14/20
316	5/10	398	3/10
282	4/10	355	2/10
251	2/10	316	0/10
223	0/10	282	0/10
158	0/10	251	0/10
Control	0/10	Control	0/10

PROBIT ANALYSIS RESULTS[(a)]

	Male	Female	Male and Female
LD_{10}	227(192-268)	341(305-380)	256(212-309)
LD_{50}	328(299-359)	440(412-470)	386(348-428)
LD_{90}	473(401-559)	566(507-636)	581(474-711)

[(a)]Doses with 95% confidence intervals are expressed in mg/kg.

part to enhanced compound absorption resulting from the higher vehicle volumes that were required to dissolve sulfone.

With the exception of sulfone, mice were more sensitive to the lethal effects of these compounds than were rats. Sulfone was moderately less toxic than sulfoxide to male and female mice and moderately more toxic than sulfide to male mice. Sulfoxide was significantly more toxic than either sulfone or sulfide to both male and female mice in this study.

ACUTE DERMAL TOXICITY STUDY IN RATS

METHODS

Dermal range finding studies were conducted in Fischer 344 rats on the following compounds: p-chlorophenyl methyl sulfone, p-chlorophenyl methyl sulfide, and p-chlorophenyl methyl sulfide. Each compound was tested at three dosage levels plus one common control group using two rats per sex per dose. Dosage levels used were 5630 mg/kg, 2190 mg/kg, and 1000 mg/kg.

The rats, supplied by Charles River Laboratories, were housed in polycarbonate cages (size 19-1/2 in. long x 10 in. wide x 6 in. high) with stainless steel barred lids, four rats per cage. Water was supplied via glass bottles with rubber stoppers placed on the top of each cage. Pelleted feed was supplied ad libitum (Wayne Laboratory Animal Diets, Lab-Blox F-6, Allied Mills, Inc., Chicago, Illinois). The rats were placed under quarantine for a period of 1 week and were observed daily for any abnormalities.

After being released from quarantine, the rats were individually housed under the conditions stated above. Each rat was given a temporary identification number and was weighed using a Mettler PT15 scale. The animals were then randomized by weight. The rats were eartagged with a permanent identification number ranging from 4821-4860, inclusive. Eartags used were #1005 Monel, size 1, supplied by National Band and Tag Co., Newport, Kentucky.

Doses were set on the basis of earlier work done on these compounds. Acetone (J. T. Baker Chemical Co., Phillipsburg, New Jersey, lot #729-2561) served as the vehicle for all three compounds and was also used as the control compound. Solutions were prepared by weighing out the required amount of each compound into a 50 ml glass volumetric flask and then adding the acetone for a total volume of 50 ml. Exceptions to this procedure were the high dose sulfoxide and sulfone solutions which had to be dissolved into 100 ml and 150 ml, respectively, due to the

large amount of compound to be dissolved. Subsequent alterations were made in dosage volumes to cover these variations.

The rats were prepared for dosing by shaving their backs from approximately the first thoracic vertebrae region to the sacral region and about midway to ground down each side. An Oster small animal electric clipper, Model A-2, was used with a size 40 blade. Clipper Mate, an aerosol to sanitize, lubricate, and cool clipper blades was used to keep the clipper cutting smoothly (Carson Chemicals Inc., New Castle, Indiana, lot #7101). The solutions were applied onto two layers of gauze pads using 3 cc and 1 cc Becton-Dickinson disposable tuberculin syringes. The first layer, which came into direct contact with the skin, was a Johnson's gauze sponge, 4 x 4, 8-ply, cut into halves (Johnson and Johnson, New Brunswick, New Jersey). The second layer, directly adjacent to the first, was composed of a plasticized Telfa surgical dressing, 4 x 3, which was separated into two pieces. Each piece was then cut into fourths. (Telfa pads were supplied by Kendall, Hospital Products Division, Chicago, Illinois.) The pads were placed onto the shaved area of the animals' backs and secured with a single layer of Elastoplast elastic adhesive bandage 2 inches wide (Beiersdorf, Inc., South Norwalk, Connecticut). The rats were then returned to their individual cages. After 24 hours, the bandages and pads were removed. A clean paper towel saturated with acetone was used to wipe any excess compound from the shaved area of each animal. Daily observations of each animal were performed for 14 days following dosing, with all animals being weighed on day 14.

RESULTS

The results of the Dermal Toxicity Study are presented in Table 7. All animals treated at the 5630 mg/kg level with sulfide died within 24 hours following removal of the bandage. No other mortalities were observed.

Pharmacotoxic symptoms were similar to those observed following an acute oral dose. The relative toxicity of the three compounds following

TABLE 7. MORTALITIES OF RATS TREATED DERMALLY WITH p-CHLOROPHENYL METHYL SULFIDE, SULFOXIDE, AND SULFONE[a]

Dose (mg/kg)	Log Dose	Compounds					
		Sulfide		Sulfoxide		Sulfone	
		Male	Female	Male	Female	Male	Female
5630	3.75	2/2	2/2	0/2	0/2	0/2	0/2
2190	3.35	0/2	0/2	0/2	0/2	0/2	0/2
1000	3.00	0/2	0/2	0/2	0/2	0/2	0/2

(a) The period of exposure was 24 hours followed by a 14-day observation period.

a 24-hour dermal exposure was sulfone, sulfoxide, and sulfide in order of increasing severity. At the 5630 mg/kg level, the animals treated with sulfide and sulfoxide showed an immediate decrease in motor activity, followed by a loss of coordination, prostration, loss of consciousness, and labored respiration; this sequence of events was followed by death in animals treated with sulfide and by a gradual recovery over a 5-day period in animals treated with sulfoxide. Both sulfide and sulfoxide produced severe lacrimation and diarrhea at the highest dosage level.

Rats treated at 2190 mg/kg level with sulfide or sulfoxide showed similar symptoms albeit less severe, to those observed at the highest level. Decreased motor activity and incoordination were noted for 7 days following treatment at the 2190 mg/kg dosage level with sulfide. Animals treated with sulfoxide at this level were normal within 24 hours following removal of the bandage.

Pharmacotoxic symptoms were not observed in any animals treated with sulfone. No toxicity was seen in rats receiving 1000 mg/kg sulfoxide. However, rats treated with 1000 mg/kg sulfide showed a decrease in locomotor activity for approximately 24 hours following compound administration.

SKIN IRRITATION STUDY IN RABBITS

METHODS

Skin irritation studies were conducted in New Zealand albino rabbits using the Modified Draize Procedure (Draize, 1959). Three male and three female rabbits were tested for each compound.

The rabbits, supplied by King's Wheel Rabbitry, arrived on July 25, 1978, and were singly housed in suspended stainless steel slotted cages (Labco Inc., Columbus, Ohio) measuring 18 in. wide x 21 in. deep x 14 in. high, grouped in a mobile stand containing nine cages (three horizontal x three vertical). Cage floors were stainless steel screening above drop pan inserts of Deotized animal cage board (The Upjohn Co., Kalamazoo, Michigan). Water was supplied ad libitum via 500 cc polycarbonate bottles with pure gum rubber stoppers and stainless steel sippers. Pelleted feed was supplied ad libitum (Purina Rabbit Chow Checkers, Ralston Purina Co., St. Louis, Missouri) in gravity feeders included in the cage construction.

The rabbits were placed under quarantine for a period of 1 week and were observed daily for any abnormalities. After being released from quarantine on August 3, 1978, the rabbits remained under the same conditions stated above. Each rabbit was given a temporary identification number and randomized by number. Several rabbits were excluded from this randomization due to ocular discharge and symptoms of respiratory disease as detailed in laboratory record book #33436. Rabbits to be used on study were then eartagged with a permanent identification number ranging from 16-33, inclusive (eartags were standard size nylon Rototags obtained from Nasco, Inc., Fort Akinson, Wisconsin).

In preparation for dosing, the rabbits' backs were shaved from approximately the first thoracic vertebrae region to the sacral region and midway down each side using an Oster small animal electric clipper, Model A-2, with size 40 blades. Clipper Mate, an aerosol to sanitize, lubricate, and cool clipper blades was used to keep the clipper cutting

smoothly (Carson Chemicals Inc., New Castle, Indiana, lot #7101). The left half of the rabbits' shaved area was abraded by lightly dragging a disconnected clipper-head across the skin.

Slurries or solutions of the compounds were prepared at 50% (W/V) concentrations using corn oil as the vehicle (Mazola corn oil, Best Foods, Englewood Cliffs, New Jersey). The test mixtures were prepared by weighing 50 g of each compound into separate 100 ml volumetric flasks. Corn oil was then added to bring the total volume up to 100 ml. The solid slurries (sulfone and sulfoxide) were stirred overnight to assure that the corn oil was saturated with compound. The sulfide was completely soluble in corn oil at 50% concentration and dissolved after mixing for a period of approximately 5 minutes.

Four patch sites were included on each animal. The two anterior patch sites served as controls, receiving corn oil only. The two posterior patch sites received the test compounds. Both corn oil and compound mixtures were applied via 1 cc tuberculin disposable syringes (Becton, Dickinson and Co., Rutherford, New Jersey) onto two layers of 1 in. square plasticized Telfa surgical dressing (Kendall, Hospital Products Division, Chicago, Illinois). The dressings were then placed onto the shaved areas of the animals' backs and secured with a double layer of Elastoplast elastic adhesive bandage 2 in. wide (Beiersdorf, Inc., South Norwalk, Connecticut). Foam-backed Elizabethan collars (Evsco Pharmaceutical Corp., Oceanside, New York) were placed around the necks of the animals to prevent them from scratching off the bandages, and the animals were returned to their cages. After 24 hours, the bandages were removed and a clean paper towel saturated with acetone (J. T. Baker Chemical Co., Phillipsburg, New Jersey) was used to wipe any excess compound from the shaved area of each animal.

Skin was graded according to the Draize system (Table 8). Skin was evaluated at 24, 48, and 72 hours posttreatment. If erythema or edema were present at the 72-hour evaluation, additional evaluations were made at 7, 14, and 21 days or until the skin returned to normal.

TABLE 8. SKIN GRADING GUIDE

	Score
Erythema and Eschar Formation	
No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined)	2
Moderate edema (raised $\sim$ 1 mm)	3
Severe edema (raised > 1 mm and extending beyond area of exposure	4

RESULTS

The results of the skin irritation study in rabbits are presented in Table 9. Erythema/eschar scores and edema scores were obtained by taking the average of the four scores recorded for abraded and intact skin at 24 and 72 hours. The primary irritation score for each animal is equal to the sum of the average erythema/eschar score and the average edema score.

Sulfide did not produce irritation in any of the rabbits tested, while sulfone resulted in mild irritation for one male and one female. In contrast, sulfoxide produced mild irritation in four rabbits and severe irritation with eschar formation in one female rabbit.

TABLE 9. SKIN IRRITATION OF p-CHLOROPHENYL METHYL SULFIDE, SULFOXIDE, AND SULFONE IN RABBITS

Compound	Sex and Animal ID	Scores		
		Erythema and Eschar Formation[a]	Edema[a]	Primary Irritation[b]
Sulfide	Male 16	0.0	0.0	0.0
"	Male 17	0.0	0.0	0.0
"	Male 18	0.0	0.0	0.0
"	Female 19	0.0	0.0	0.0
"	Female 20	0.0	0.0	0.0
"	Female 21	0.0	0.0	0.0
Sulfone	Male 22	0.0	0.0	0.0
"	Male 23	0.0	0.0	0.0
"	Male 24	0.5	0.0	0.5
"	Female 25	0.0	0.0	0.0
"	Female 26	0.0	0.0	0.0
"	Female 27	1.0	0.0	1.0
Sulfoxide	Male 28	0.0	0.0	0.0
"	Male 29	1.0	0.0	1.0
"	Male 30	0.5	0.0	0.5
"	Female 31	0.5	0.0	0.5
"	Female 32	4.0	0.5	4.5
"	Female 33	1.0	0.0	1.0

(a) Values represent an average of four scores graded for abraded and intact skin at 24 and 72 hours (maximum score = 4).

(b) Primary irritation is the sum of the average erythema/eschar and edema scores (maximum score = 8).

EYE IRRITATION STUDY IN RABBITS

METHODS

Eye irritation studies were conducted in New Zealand albino rabbits using the Modified Draize Procedure (Draize, 1959). Five male rabbits were tested for each compound.

The rabbits, supplied by King's Wheel Rabbitry, arrived on July 25, 1978, and were singly housed in suspended stainless steel slotted cages (Labco, Inc., Columbus, Ohio) measuring 18 in. wide x 21 in. deep x 14 in. high, grouped in a mobile stand containing nine cages (three horizontal x three vertical). Cage floors were stainless steel screening above drop pan inserts of Deotized animal cage board (The Upjohn Co., Kalamazoo, Michigan). Water was supplied ad libitum via 500 cc polycarbonate bottles with pure gum rubber stoppers and stainless steel sippers. Pelleted feed was supplied ad libitum (Purina Rabbit Chow Checkers, Ralston Purina Co., St. Louis, Missouri) in gravity feeders included in the cage construction.

The rabbits were placed under quarantine for a period of 1 week and were observed daily for any abnormalities. After being released from quarantine on August 3, 1978, the rabbits remained under the same conditions stated above. Each rabbit was given a temporary identification number and randomized by number according to the method of Goldstein (1964). Several rabbits were excluded from this randomization due to ocular discharge and symptoms of respiratory disease as detailed in laboratory record book #33436, page 49. Rabbits to be used on study were eartagged with a permanent identification number ranging from 1-15, inclusive (eartags were standard size nylon Rototags obtained from Nasco, Inc., Fort Atkinson, Wisconsin).

Prior to treatment, both eyes of each rabbit were examined using a slit-lamp to determine the condition of the conjunctivae, cornea, iris, and retina and to search for particles floating in the anterior chamber. The dye fluoroscein was used to make visible any erosion of the epithelial

TABLE 10. DRAIZE OCULAR IRRITATION SCORE SYSTEM

		Pretest	2 Hr	24 Hr	48 Hr	72 Hr
Cornea (A)						
No ulceration or opacity		0	0	0	0	0
Scattered or diffuse areas of opacity, details of iris clearly visible		(1)	(1)	(1)	(1)	(1)
Easily discernible translucent areas, details of iris slightly obscured		2	2	2	2	2
Opalescent areas, no details of iris visible, size of pupil barely discernible		3	3	3	3	3
Complete corneal opacity, iris invisible		4	4	4	4	4
Corneal score AX5	Total					
Iris (B)						
Normal		0	0	0	0	0
Marked folds, congestion, swelling, circumcorneal injection (any of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)		(1)	(1)	(1)	(1)	(1)
No reaction to light, hemorrhage, gross destruction (any or all of these)		2	2	2	2	2
Iris score BX5	Total					
Conjunctivae						
(C) Redness						
Vessels normal		0	0	0	0	0
Vessels definitely injected above normal		1	1	1	1	1
Diffuse crimson red, individual vessels not easily discernible		(2)	(2)	(2)	(2)	(2)
Diffuse beefy red		3	3	3	3	3
(D) Chemosis						
No swelling		0	0	0	0	0
Any swelling above normal (including nicitating membrane)		1	1	1	1	1
Obvious swelling with partial evasion of lids		(2)	(2)	(2)	(2)	(2)
Swelling with lids about half-closed		3	3	3	3	3
Swelling with lids more than half-closed		4	4	4	4	4
Conjunctival score (C+D)X2	Total					
	Total Eye Score	____	____	____	____	____

layer. The fluoroscein was flushed out of the eyes with physiological saline. All eyes were scored according to the Draize method (Table 10).

The right eye of each animal served as the control, undergoing no treatment. The left eye served as the test eye. Compound amounts used for the tests were 0.1 ml for sulfide, the only liquid, and 100 mg for the solids sulfone and sulfoxide. The crystals of the solid compounds were crushed to a fine powder using a mortar and pestle.

Compounds were applied to the conjunctival sac after pulling the left lower eyelid away from the eye. After application, the eyelids were held shut for approximately 10 seconds. The rabbits were restrained for 2 hours and were again examined for ocular abnormalities. After the examination, they were returned to their cages. Additional examinations were performed at 24, 48, and 72 hours and at 7 days following treatment. If abnormalities were found during the examination made at 7 days, the rabbit was examined again at 14 days and 21 days.

RESULTS

The results of the eye irritation study in rabbits are given in Table 11. An animal was considered as exhibiting a positive reaction if the test substance produced at any of the readings ulceration of the cornea (other than a fine stippling), or opacity of the cornea (other than a slight dulling of the normal luster), or inflammation of the iris (other than a slight deepening of the folds (or rugae) or a slight circumcorneal injection of the blood vessels), or if such substance produced in the conjunctivae (excluding the cornea and iris) an obvious swelling with partial eversion of the lids or a diffuse crimson-red with individual vessels not easily discernible. The test was considered positive if four or more of the animals in the test group exhibited a positive reaction.

Animals treated with sulfone did not exhibit positive responses at any examination. Only very mild swelling and redness of the conjunctiva were noted for three animals. The test of eye irritation for sulfone was considered to be negative.

TABLE 11. EYE IRRITATION OF p-CHLOROPHENYL METHYL SULFIDE, SULFOXIDE, AND SULFONE IN RABBITS

Compound	Sex and Animal ID	Test Results (a)			
		Cornea	Iris	Conjunctivae-Redness	Conjunctivae-Chemosis
Sulfide	Male 1	+ (72 hrs)	+ (24 hrs)	+ (24 hrs)	Negative
"	Male 2	+ (24 hrs)	Negative	Negative	Negative
"	Male 3	Negative	Negative	Negative	Negative
"	Male 4	+ (24 hrs)	Negative	Negative	+ (2 hrs)
"	Male 5	+ (24 hrs)	Negative	+ (2 hrs)	+ (2 hrs)
Sulfone	Male 6	Negative	Negative	Negative	Negative
"	Male 7	Negative	Negative	Negative	Negative
"	Male 8	Negative	Negative	Negative	Negative
"	Male 9	Negative	Negative	Negative	Negative
"	Male 10	Negative	Negative	Negative	Negative
Sulfoxide	Male 11	+ (21 days)	+ (24 hrs)	+ (48 hrs)	+ (48 hrs)
"	Male 12	+ (14 days)	+ (2 hrs)	+ (72 hrs)	+ (48 hrs)
"	Male 13	+ (21 days)	+ (48 hrs)	+ (72 hrs)	+ (72 hrs)
"	Male 14	+ (21 days)	+ (72 hrs)	+ (72 hrs)	+ (72 hrs)
"	Male 15	+ (21 days)	+ (24 hrs)	+ (72 hrs)	+ (72 hrs)

(a) Time of observation at which a positive response was last observed is indicated in parenthesis.

Sulfide produced positive corneal responses in four out of five animals treated. All eyes treated with sulfide were normal at the 7-day examination indicating complete reversibility of the lesions. The test of eye irritation for sulfide was considered to be positive; however, the lesions were reversible.

All animals treated with sulfoxide exhibited positive responses for the cornea, iris, and conjunctivae. Lesions of the iris and conjunctivae were completely reversible after 7 days. Significant corneal opacity was observed in four out of five animals treated with sulfoxide throughout the 21-day observation period. The test of eye irritation for sulfoxide was considered to be positive with the production of irreversible corneal lesions.

SKIN SENSITIZATION STUDIES IN GUINEA PIGS

METHODS

Female Hartley albino guinea pigs (Charles River Breeding Laboratories, Wilmington, Massachusetts) weighing 300-500 grams were used in the skin sensitization tests. Each of the three test groups (one group per compound) and the control group contained eight guinea pigs.

The guinea pigs were housed in polycarbonate cages (size 19-1/2 in. long x 10 in. wide x 6 in. high) with stainless steel barred lids, one guinea pig per cage. Water was supplied via glass bottles with rubber stoppers placed on the top of each cage. Pelleted feed was supplied ad libitum (Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Missouri). The guinea pigs were placed under quarantine for a period of 1 week and were observed daily for any abnormalities.

After being released from quarantine, the guinea pigs were individually housed under the same conditions stated above. Each guinea pig was given a temporary identification number and was weighed using a Mettler PT15 scale. The animals were then randomized according to the method of Goldstein (1964). The guinea pigs were identified by means of earpunching and cage cards.

The intradermal injections were made with the compounds incorporated in Freund's Complete Adjuvant (Difco Laboratories, Detroit, Michigan). Immediately before injection, an emulsion was prepared by blending the commercial adjuvant with an equal volume of water. The adjuvant was placed in a container and the aqueous phase was added in several installments while homogenizing with a rotating stirrer. The oil-soluble test chemicals were dissolved or suspended in the adjuvant (a mixture of paraffin oil and an emulsifier with killed mycobacteria). The final concentration of the allergen was 5% by weight. The injections did not produce local necrosis or ulceration and were sufficiently free of systemic toxicity as not to impair the health of the animal. The

test chemicals which were to be injected without adjuvant were incorporated into corn oil to give 5% (W/V) final concentrations.

For the topical application, the test chemicals were finely pulverized and incorporated into petrolatum at a 25% (W/V) concentration. A mild to moderate irritation was produced with this concentration.

Induction Procedure

Induction was a two-stage operation. First, three pairs of injections were made simultaneously. Second, closed patch exposure was performed over the injection sites 1 week later. The shoulder region was the induction site. An area 4 x 6 cm was clipped short with an Oster Model A-2 clipper (Oster Corporation, Milwaukee, Wisconsin).

Intradermal Injections

Two rows of three injections were made, one on each side of the midline as follows: (1) 0.1 ml of the adjuvant without the test agent, (2) 0.1 ml of test agent without adjuvant, and (3) 0.1 ml of the test substance emulsified in complete adjuvant. The injection sites were within the boundaries of the 2 x 4 cm patch, which was applied 1 week later.

Topical Application

One week after the injections, the same area was clipped and shaved closely with an Oster clipper. The area was pretreated with 10% sodium lauryl sulfate (SLS) in petrolatum 24 hours before the patch was applied. The SLS was massaged into the skin with a glass rod; no bandage was applied. This concentration of SLS enhances sensitization by provoking a mild inflammatory reaction.

The test agent in petrolatum was spread over a 2 x 4 patch of Whatman No. 3 mm filter paper (Scientific Products, Obetz, Ohio) in a thick, even layer. The patch was covered by overlapping an impermeable Telfa plasticized pad (Kendall, Chicago, Illinois). This in turn was

firmly secured by an Elastoplast elastic adhesive bandage (Beiersdorf, Inc., South Norwalk, Connecticut) wound around the torso of the animal. This dressing was left in place for 48 hours.

Challenge Procedure

Challenge was by topical application. The solids were incorporated in petrolatum at 25% (W/V) concentration, and the liquid (sulfide) was used undiluted. The animals were challenged 2 weeks after topical induction. Hair was removed from a 5 x 5 cm area on the flank by clipping and shaving as before. The test agent was applied on a 2 x 2 cm piece of filter paper in the same fashion as for topical induction. The patch was sealed to the flank for 24 hours under a 4-cm strip of 1-1/2 inch Telfa. This in turn was secured by Elastoplast wound around the trunk. The dressing was secured in a fashion which afforded complete occulsion.

The challenge site was evaluated 24 hours after removal of the patch. The sites were again examined in an additional 24 hours (48 hours following challenge) mainly to detect weak, slowly developing reactions. Three hours prior to the first reading, the test site was shaved with the electric razor and the skin was gently cleansed of excess chemical with ether. Redness constituted the minimum criterion of an allergic reaction. The reactions were scored on a four-point scale: no reaction, 0; scattered mild redness, 1; moderate and diffuse redness, 2; intense redness and swelling, 3. Skin sections from the challenge areas were collected for histopathological evaluation.

RESULTS

Changes were observed in the skin of the guinea pigs treated with sulfone. These changes were characterized by minimal focal acantholysis in one animal and minimal focal intracellular vacuolation in two animals (Tables 12 and 13).

TABLE 12. FREQUENCY OF LESIONS OCCURRING IN GUINEA PIGS IN THE SKIN SENSITIZATION STUDY

Lesions	Treatments			
	Control	Sulfone	Sulfoxide	Sulfide
Epidermis:				
Mild, focal acantholysis	0	1	0	0
Mild, focal, intracellular vacuolation	0	2	1	
Mild, multifocal, intracellular vacuolation	0	0	1	0
Dermis:				
Chronic, multifocal, papillary dermatitis	0	0	1	0

TABLE 13. INCIDENCE OF LESIONS OBSERVED IN GUINEA PIGS IN THE SKIN SENSITIZATION STUDY

Treatment	Lesion	Animal Number
Control	None	--
Sulfone	Epidermis, mild, focal acantholysis	78N1474
	Epidermis, mild, focal, intracellular vacuolation	78N1474 78N1478
Sulfoxide	Epidermis, mild, focal, intracellular vacuolation	78N1714 78N1715
	Dermis, chronic, mild, multifocal papillary dermatitis	78N1714
Sulfide	None	--

Changes were observed in the skin of the guinea pigs treated with sulfoxide. These changes included minimal focal intracellular vacuolation in two animals and mild multifocal papillary dermatitis in one animal (Tables 12 and 13).

Changes were not observed in the skin of the guinea pigs treated with sulfide.

The changes observed in the animals treated with sulfone and sulfoxide were infrequently observed. The minimal and focal nature of the changes does not strongly support the conclusion that they were drug induced, but they probably represent background changes not infrequently encountered in laboratory-reared animals.

28-DAY RANGE FINDING STUDIES IN RATS AND MICE

METHODS

Sulfone, sulfide, and sulfoxide were expected to be unpalatable when mixed with feed in high concentrations. The undesirable taste and odor of the compounds was considered to be a potential problem in establishing target organ toxicity via a feeding study. Hence, initial efforts in the range-finding studies were directed toward masking the taste and odor of the compounds. Preliminary trials were unsuccessful in finding a suitable agent for this purpose and rats rejected feed containing high concentrations of test materials. Mice were more tolerant of test diets than rats. Both 28-day feeding studies and 28-day gavage studies were conducted for all three compounds in rats to determine the optimum route of exposure.

RESULTS

Mortality, body weight, and food consumption data for rats in the 28-day feeding study are presented in Tables 14-19. Tests with sulfone and sulfide were conducted at approximately the same time, while the sulfoxide study started at a later date due to delays in receiving the compound.

All rats receiving diets containing 9000 ppm sulfide died within the first week of treatment. Essentially no feed was consumed by rats at this level and all deaths were attributed to starvation. No other rats died that were treated with sulfide- or sulfone-dosed feed. Experimental difficulties were encountered with the common control group used for sulfide and sulfone. Body weight and food consumption data were compared to a control group of Fischer 344 rats used in a similar feeding study. Generally, body weight and food consumption were depressed in a dose-related manner for rats treated with sulfide and sulfone. Food consumption

TABLE 14. BODY WEIGHTS OF FISCHER 344 RATS IN THE 28-DAY SULFONE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Days on Test[a] 0	7	14	21	28	Δ Body Weight (Percent Control)
			Male				
2250	0/5	210	201	196	193	200	-30
562	0/5	226	212	235	215	244	48
281	0/5	240	240	261	270	267	82
Control[b]	0/5	231	239	241	246	264	-
			Female				
2250	0/5	135	129	219	141	142	29
562	0/5	133	141	148	154	156	96
281	0/5	155	159	162	168	172	71
Control[b]	0/5	138	143	148	161	162	-

(a) Values are group means expressed in grams.

(b) These data were obtained from a control group of Fischer 344 rats used in a similar feeding study.

TABLE 15. BODY WEIGHTS OF FISCHER 344 RATS IN THE 28-DAY SULFIDE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Days on Test[a] 0	7	14	21	28	Δ Body Weight (Percent Control)
			Male				
9000	5/5	-	-	-	-	-	-
2250	0/5	210	183	187	183	193	-52
562	0/5	214	209	217	205	232	58
281	0/5	235	240	258	267	267	97
Control[b]	0/5	231	239	241	246	264	-
			Female				
9000	5/5	-	-	-	-	-	-
2250	0/5	133	119	129	143	143	42
562	0/5	133	139	146	151	152	75
281	0/5	150	158	157	160	168	75
Control[b]	0/5	138	143	148	161	162	-

(a) Values are group means expressed in grams.

(b) These data were obtained from a control group of Fischer 344 rats used in a similar feeding study.

TABLE 16. BODY WEIGHTS OF FISCHER 344 RATS IN THE 28-DAY SULFOXIDE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Days on Test(a) 0	7	14	21	28	Δ Body Weight (Percent Control)
			Male				
5200	4/5	129	100	93	94	124	-4.5
2600	0/5	123	115	129	148	162	35
1300	0/5	132	150	169	187	206	67
650	0/5	124	151	171	195	210	78
325	0/5	125	158	181	202	227	93
162	0/5	125	162	188	210	232	97
Control	0/10	126	161	190	214	236	-
			Female				
5200	3/5	103	82	76	80	93	-22
2600	0/5	104	98	105	110	120	35
1300	0/5	103	112	122	129	135	70
650	0/5	105	117	127	136	146	89
325	0/5	103	116	125	136	145	91
162	0/5	102	120	128	138	146	96
Control	0/10	107	124	134	143	153	-

(a) Values are group means expressed in grams.

TABLE 17. WEEKLY FEED CONSUMPTIONS OF FISCHER 344 RATS IN THE 28-DAY SULFONE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Week(a) 1	2	3	4	Mean Weekly Feed Consumption (Percent Control)
		Male				
2250	0/5	81	66	99	74	59
562	0/5	94	97	100	104	73
281	0/5	99	118	107	122	82
Control(b)	0/5	132	153	126	135	-
		Female				
2250	0/5	49	43	78	75	63
562	0/5	64	57	83	74	72
281	0/5	59	77	87	96	82
Control(b)	0/5	84	91	100	112	-

(a) Values are group means expressed in grams.

(b) These data were obtained from a control group of Fischer 344 rats used in a similar feeding study.

TABLE 18. WEEKLY FEED CONSUMPTIONS OF FISCHER 344 RATS IN THE 28-DAY SULFIDE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Week(a) 1	2	3	4	Mean Weekly Feed Consumption (Percent Control)
		Male				
9000	5/5	-	-	-	-	-
2250	0/5	49	69	100	82	56
562	0/5	95	80	90	94	66
281	0/5	99	112	105	106	77
Control(b)	0/5	132	153	126	135	-
		Female				
9000	0/5	-	-	-	-	-
2250	0/5	34	54	91	79	65
562	0/5	60	61	83	69	70
281	0/5	58	65	74	91	74
Control(b)	0/5	84	91	100	112	-

(a) Values are group means expressed in grams.

(b) These data were obtained from a control group of Fischer 344 rats used in a similar feeding study.

TABLE 19. WEEKLY FEED CONSUMPTIONS OF FISCHER 344 RATS IN THE 28-DAY SULFOXIDE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Week(a) 1	2	3	4	Mean Weekly Feed Consumption (Percent Control)
		Males				
5200	4/5	30	64	65	63	50
2600	0/5	55	84	104	88	78
1300	0/5	79	107	122	107	93
650	0/5	83	102	123	106	93
325	0/5	86	114	137	114	101
162	0/5	99	110	135	119	104
Control	0/10	98	108	121	119	-
		Females				
5200	3/5	32	55	65	50	59
2600	0/5	42	70	83	67	78
1300	0/5	59	74	95	76	88
650	0/5	65	76	97	76	91
325	0/5	69	78	100	86	97
162	0/5	78	77	100	80	98
Control	0/10	77	82	99	84	-

(a) Values are group means expressed in grams.

was most severely affected during the first 2 weeks and approached control values at lower doses during weeks 3 and 4. However, differences in initial body weights made interpretation of the sulfide and sulfone data difficult.

For rats treated with feed containing 5200 ppm sulfoxide (Tables 20-22), 80% mortality was observed in males and 60% mortality was observed in females. There were no mortalities in any other groups during the 28 days of treatment. A depression in food consumption was noted in all groups for week 1, except for the 162 ppm dose. In animals receiving 1300 ppm or less, food consumption returned approximately to control values during weeks 2, 3, and 4 for males and during weeks 3 and 4 for females. Food consumption remained depressed throughout the study for rats receiving 5200 or 2600 ppm sulfoxide. Body weights were more severely affected during the first 2 weeks of treatment. The relative decrement in weight gain decreased as the study progressed, except at the 5200 ppm treatment level.

Body weight, food consumption, and mortality data for rats in the 28-day oral gavage study are given in Tables 23-25.

Mortalities in groups of rats treated with sulfide occurred in a frequency similar to those treated with sulfone. Female rats exhibited a higher rate of mortality for both compounds.

All rats treated with 400 mg/kg sulfoxide died within the first 4 days of treatment and one female rat in the 200 mg/kg group died during the final week of treatment. These deaths were considered to be compound related. Three female rats died during the first week of treatment; one death resulted from traumatic injuries and causes for the other two were not apparent at necropsy.

In the first phase of the gavage study, rats treated with sulfone received corn oil vehicle at a rate of 28 ml/kg. This was done in an effort to administer sulfone at higher doses as a solution rather than as a suspension. However, the high mortality observed at all dosage levels (100% at 100 mg/kg or above and 60% at 50 mg/kg) required that additional animals be placed on study at lower dosage levels. This permitted a lower volume of vehicle administration (5 ml/kg) for subsequent groups tested.

TABLE 20. BODY WEIGHTS OF FISCHER 344 RATS IN THE 28-DAY SULFONE ORAL GAVAGE STUDY (RANGE FINDING)

Group[a]	Dose (mg/kg/day)	Mortality	Days on Test[b] 0	7	14	21	28	Δ Body Weights (Percent Control)
			Male					
1	460	5/5	-	-	-	-	-	-
2	200	5/5	-	-	-	-	-	-
3	100	5/5	-	-	-	-	-	-
4	50	3/5	230	205	213	230	221	-22
5	Control	0/5	201	204	223	228	241	-
6	100	0/6	202	179	213	229	240	95
7	50	0/5	198	201	226	238	247	122
8	25	0/5	195	212	223	235	244	122
9	12.5	0/5	192	205	221	231	240	120
10	6.25	0/5	197	221	236	246	256	148
11	3.12	0/5	209	224	241	253	262	128
			Female					
1	320	5/5	-	-	-	-	-	-
2	200	5/5	-	-	-	-	-	-
3	100	5/5	-	-	-	-	-	-
4	50	3/5	152	133	159	162	162	50
5	Control	0/5	160	169	169	177	180	-
6	100	5/5	-	-	-	-	-	-
7	50	3/5	158	118	131	160	176	90
8	25	0/5	160	141	150	166	176	80
9	12.5	0/5	155	162	165	169	174	95
10	6.25	0/5	161	161	161	167	169	40
11	3.12	0/5	158	162	166	167	172	70

(a) Groups 1-5 and Groups 6-11 were treated at a rate of 28 ml/kg and 5 ml/kg, respectively.

(b) Values are group means expressed in grams.

TABLE 21. BODY WEIGHTS OF FISCHER 344 RATS IN THE 28-DAY SULFIDE ORAL GAVAGE STUDY (RANGE FINDING)

Dose (mg/kg/day)	Mortality	Days on Test[a] 0	7	14	21	28	Δ Body Weight (Percent Control)
			Male				
530	5/5		-	-	-	-	
265	5/5		-	-	-	-	
220	5/5		-	-	-	-	
180	1/5	194	173	192	202	216	55
150	1/5[b]	215	180	187	200	215	0
75	0/5	214	205	215	240	247	82
37.5	0/5	190	203	220	237	246	140
18.8	0/5	199	211	224	235	244	112
Control[c]	0/5	201	204	223	228	241	-
			Female				
330	5/5	-	-	-	-	-	
150	2/5	147	133	159	162	164	85
75	2/5	139	143	154	146	159	55
37.5	2/5	147	137	150	159	155	40
18.8	0/5	156	158	166	166	173	85
9.4	0/5	160	160	162	168	170	50
Control[c]	0/5	160	169	169	177	180	-

(a) Values are group means expressed in grams. (All treatment groups dosed at a rate of 5.0 ml/kg.)

(b) Animal died from aspiration of dosing solution.

(c) Control group common with the sulfone-treated animals that received corn oil vehicle at a rate of 28 ml/kg.

TABLE 22. BODY WEIGHTS OF FISCHER 344 RATS IN THE 28-DAY SULFOXIDE ORAL GAVAGE STUDY (RANGE FINDING)

Dose (mg/kg/day)	Mortality	Days on Test[a] 0	7	14	21	28	Δ Body Weight (Percent Control)
				Male			
400	5/5	-	-	-	-	-	-
200	0/5	135	131	164	173	173	42
100	0/5	130	152	166	180	198	75
50	0/5	131	161	181	188	212	89
25	0/5	135	167	186	198	219	92
12.5	0/5	125	154	177	192	212	96
6.25	0/5	134	171	196	210	229	104
Control	0/10	134	167	190	204	225	-
				Female			
400	5/5	-	-	-	-	-	-
200	1/5	102	105	121	117	133[b]	82
100	0/5	109	120	128	131	140	82
50	0/5	107	124	131	133	142	92
25	0/5	110	124	134	137	145	92
12.5	0/5	107	120	130	135	143	95
6.25	0/5	109	126	135	140	151	110
Control	3/10	106	119	128	133	144	-

(a) Values are group means expressed in grams.

(b) N = 4.

TABLE 23. WEEKLY FEED CONSUMPTIONS OF FISCHER 344 RATS IN THE 28-DAY SULFONE ORAL GAVAGE STUDY (RANGE FINDING)

Group[a]	Dose (mg/kg/day)	Mortality	Week[b] 1	2	3	4	Mean Weekly Feed Consumption (Percent Control)
			Male				
1	460	5/5	-	-	-	-	-
2	200	5/5	-	-	-	-	-
3	100	5/5	-	-	-	-	-
4	50	3/5	27	43	62	47	77
5	Control	0/5	48	80	55	48	-
6	100	0/5	52	98	129	120	165
7	50	0/5	78	95	124	120	181
8	25	0/5	89	78	104	124	170
9	12.5	0/5	90	92	106	117	175
10	6.25	0/5	83	80	113	113	193
11	3.12	0/5	108	101	108	130	185
			Female				
1	320	5/5	-	-	-	-	-
2	200	5/5	-	-	-	-	-
3	100	5/5	-	-	-	-	-
4	50	3/5	20	31	46	27	-
5	Control	0/5	30	47	39	33	-
6	100	5/5	-	-	-	-	-
7	50	3/5	27	30	99	113	172
8	25	0/5	46	58	88	99	197
9	12.5	0/5	67	60	66	78	184
10	6.25	0/5	65	56	78	79	187
11	3.12	0/5	55	55	61	75	166

(a) Groups 1-5 and Groups 6-11 were treated at a rate of 28 ml/kg and 5 ml/kg, respectively.

(b) Values are group means expressed in grams.

TABLE 24. WEEKLY FEED CONSUMPTIONS OF FISCHER 344 RATS IN THE 28-DAY SULFIDE ORAL GAVAGE STUDY (RANGE FINDING)

Dose (mg/kg/day)	Mortality	Week[a] 1	2	3	4	Mean Weekly Feed Consumption (Percent Control)
		Male				
530	5/5					
265	5/5					
220	5/5					
180	1/5	46	94	111	127	162
150	1/5[b]	(c)	79	97	102	160
75	0/5	69	118	124	94	175
37.5	0/5	86	98	101	130	167
18.8	0/5	93	97	99	119	179
Control[d]	0/5	48	80	55	48	-
		Female				
330	5/5	-	-	-	-	
150	2/5	(c)	85	92	72	224
75	2/5	56	88	85	77	208
37.5	2/5	34	66	90	65	172
18.8	0/5	68	56	58	82	178
9.4	0/5	61	54	71	74	176
Control[d]	0/5	30	47	39	33	-

(a) Values are group means expressed in grams.

(b) Animal died from aspiration of dosing solution.

(c) Data not recorded.

(d) Control group common with the sulfone-treated animals; received corn oil vehicle at a rate of 28 ml/kg.

TABLE 25. WEEKLY FEED CONSUMPTIONS OF FISCHER 344 RATS IN THE 28-DAY SULFOXIDE ORAL GAVAGE STUDY (RANGE FINDING)

Dose (mg/kg/day)	Mortality	Week(a) 1	2	3	4	Mean Weekly Feed Consumption (Percent Control)
			Male			
400	5/5	-	-	-	-	-
200	0/5	51	101	95	83	82
100	0/5	80	103	99	94	98
50.0	0/5	92	103	94	101	102
25.0	0/5	94	97	94	92	98
12.5	0/5	86	97	92	84	93
6.25	0/5	98	107	100	89	126
Control	0/10	93	102	102	88	-
			Female			
400	5/5	-	-	-	-	-
200	1/5	49	82	61	66	94
100	0/5	67	75	69	68	101
50.0	0/5	80	78	67	68	106
25.0	0/5	70	72	63	63	97
12.5	0/5	72	83	69	58	102
6.25	0/5	75	78	74	69	107
Control	3/10	71	71	80	57	-

(a) Values are group means expressed in grams.

Pharmacotoxic symptoms were similar for all three compounds in the gavage study. Animals receiving doses greater than 50 mg/kg responded with an immediate and marked depression in motor activity following each daily treatment. Rats receiving high doses became progressively depressed with each subsequent dose until death. Symptoms of depression completely disappeared prior to the termination of the study in all rats surviving 28 days of treatment. The pattern of symptoms prior to death was identical to that observed in animals following treatment with a single dose in the acute oral toxicity (LD_{50}) studies.

During the second phase of the gavage study, dosage levels of 100 and 50 mg/kg of sulfone were repeated and additional (lower) dosage levels were added. Mortalities among female rats were identical at both vehicle volumes for both 50 and 100 mg/kg levels. However, male rats responded quite differently at the 5 ml/kg rate in that no deaths were recorded at either 50 or 100 mg/kg levels. This difference in toxicity with larger vehicle volumes could be due to enhanced absorption of compound at lower concentrations or the increased likelihood of aspiration of dosing solution. Biotransport of sulfone could be increased by the increased plasma lipid concentration following administration of a large volume of corn oil. However, this reasoning does not explain why females responded differently than males.

Body weights and food consumption of rats treated with sulfone or sulfide are difficult to interpret due to a depression in food consumption for control rats receiving 28 ml/kg corn oil. Most treatment animals consumed greater amounts of feed than did control rats.

Body weight data for rats treated with sulfoxide show dose-related decreases for both sexes. Weekly weight gains, however, were approaching control values during the final week of the study indicating tolerance toward treatment. Mean weekly food consumption of animals treated with doses of 100 ml/kg or higher were moderately reduced. Weekly food consumption for several treatment groups exceeded control groups on several occasions.

Results of the 28-day range-finding feeding study in mice are presented in Tables 26-31. One female mouse died in the 4500 ppm sulfide

TABLE 26. WEEKLY FEED CONSUMPTIONS OF $B_3C_6F_1$ MICE IN THE 28-DAY SULFONE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Week(a) 1	2	3	4	Mean Weekly Feed Consumption (Percent Control)
			Male			
4500	0/5	33.4	19.5	16.8	45.6	63
2250	0/5	38.6	33.6	43.0	34.0	84
562	0/4	47.4	28.3	40.0	45.8	93
281	0/5	38.4	40.5	50.0	38.6	93
Control	0/5	43.4	44.6	42.0	47.2	-
			Female			
4500	0/5	25.0	11.6	9.0	39.6	44
2250	0/5	32.2	21.2	42.8	47.8	74
562	0/5	42.4	24.4	42.0	47.0	81
281	0/5	37.2	44.8	41.8	31.6	67
Control	0/5	44.8	57.0	51.6	40.4	-

(a) Values are group means expressed in grams.

TABLE 27. WEEKLY FEED CONSUMPTIONS OF $B_3C_6F_1$ MICE IN THE 28-DAY SULFIDE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Week(a) 1	2	3	4	Mean Weekly Feed Consumption (Percent Control)
			Male			
4500	0/4	16.2	40.5	37.0	42.3	77
2750	0/5	24.2	27.4	29.0	36.0	66
562	0/5	36.6	28.2	37.2	35.0	77
281	0/5	35.6	41.2	44.6	30.0	85
Control	0/5	43.4	44.6	42.0	47.2	-
			Female			
4500	1/5	16.2	26.8	29.5	30.5	53
2250	0/5	28.2	17.0	31.8	43.8	63
562	0/5	40.6	26.2	42.2	47.8	81
281	0/5	42.0	47.2	54.2	29.2	89
Control	0/5	44.8	57.0	51.6	40.4	-

(a) Values are group means expressed in grams.

TABLE 28. WEEKLY FEED CONSUMPTIONS OF $B_3C_6F_1$ MICE IN THE 28-DAY SULFOXIDE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Week(a) 1	2	3	4	Mean Weekly Feed Consumption (Percent Control)
			Male			
5200	0/5	12.0	20.6	31.2	35.0	78
2600	0/5	13.6	33.8	31.4	36.6	91
1300	0/5	33.8	40.0	39.2	39.0	130
750	0/5	20.2	37.5	36.2	39.0	107
375	0/5	28.0	44.6	40.0	47.2	132
188	0/5	26.0	46.0	40.2	48.4	131
Control	0/5	18.4	37.8	35.6	32.3	-
			Female			
5200	2/5	13.2	30.7	35.7	32.7	81
2600	0/5	21.6	32.4	33.2	30.6	88
1300	0/5	24.8	33.8	39.4	42.6	104
750	0/5	13.2	37.5	35.6	37.8	89
375	0/5	31.0	46.0	43.2	48.4	126
188	0/5	26.8	42.4	37.6	41.8	111
Control	0/5	23.5	37.3	39.5	34.1	-

(a) Values are group means expressed in grams.

TABLE 29. BODY WEIGHTS OF $B_3C_6F_1$ MICE IN THE 28-DAY SULFONE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Days on Test(a) 0	7	14	21	28	Δ Body Weight (Percent Control)
			Male				
4500	0/5	20.8	20.0	21.8	22.2	22.6	180
2250	0/5	24.9	24.4	25.2	24.6	24.6	-30
562	0/4	25.0	25.4	25.8	25.5	25.8	80
281	0/5	25.6	25.8	27.2	25.8	26.3	70
Control	0/5	25.0	26.0	25.6	25.4	26.0	-
			Female				
4500	0/5	18.4	16.6	16.8	18.6	21.0	118
2250	0/5	21.9	21.6	21.4	21.7	22.0	4
562	0/5	20.9	23.2	22.4	23.4	23.4	113
281	0/5	21.4	21.4	23.2	22.8	22.5	50
Control	0/5	21.6	22.4	23.2	23.0	23.8	-

(a) Values are group means expressed in grams.

TABLE 30. BODY WEIGHTS OF $B_3C_6F_1$ MICE IN THE 28-DAY SULFIDE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Days on Test[a] 0	7	14	21	28	Δ Body Weight (Percent Control)
				Male			
4500	0/4	24.5	22.8	25.3	25.3	25.2	70
2250	0/5	22.8	24.1	23.5	25.2	24.2	140
562	0/5	25.2	26.4	22.8	25.0	24.0[b]	-120
281	0/5	23.6	23.8	25.0	24.4	24.2	60
Control	0/5	25.0	26.0	25.6	25.4	26.0	-
				Female			
4500	1/5	21.8	20.4	22.3	22.5	21.2	-18
2250	0/4	21.2	23.0	18.3	23.5	21.2	0
562	0/5	22.6	23.0	20.3	22.8	22.0	-27
281	0/5	22.4	21.6	23.4	23.8	24.0	73
Control	0/5	21.6	22.4	23.2	23.0	23.8	-

(a) Values are group means expressed in grams.

(b) N = 3.

TABLE 31. BODY WEIGHTS OF $B_3C_6F_1$ MICE IN THE 28-DAY SULFOXIDE FEEDING STUDY (RANGE FINDING)

Concentration in Feed (ppm)	Mortality	Days on Test[a] 0	7	14	21	28	Δ Body Weight (Percent Control)
			Male				
5200	0/5	20.0	21.1	20.5	22.7	23.3	65
2600	0/5	20.5	20.3	23.7	22.1	22.4	51
1300	0/5	21.9	22.4	23.6	23.6	24.3	65
750	0/5	20.0	20.3	21.8	22.8	22.9	78
375	0/5	20.8	22.3	23.8	24.6	24.8	108
188	0/5	20.3	21.7	23.4	24.5	24.5	114
Control	0/10	20.6	21.7	22.7	24.3	24.3	-
			Female				
5200	2/5	18.3	15.3	18.4	20.9	21.7	97
2600	0/5	17.5	16.8	17.6	19.2	20.6	86
1300	0/5	17.4	17.1	18.3	18.8	19.9	71
750	0/5	18.2	18.3	19.5	20.4	20.8	74
375	0/5	17.8	18.4	19.5	20.6	21.3	100
188	0/5	18.9	19.4	20.5	21.6	22.0	89
Control	0.10	17.8	17.7	19.8	20.4	21.3	-

(a) Values are group means expressed in grams.

group and two female mice died during the first week of treatment in the 5200 ppm sulfoxide group. No other mortalities were observed. Prior to death, mice showed marked depressions in motor activity, body weight, and food consumption. Body weight gain data are difficult to interpret for mice, partially due to differences in initial body weights. In general, mice treated with sulfoxide at 750 ppm or higher showed decrements in body weights. Food consumption data for mice did not show consistent trends for any of the three compounds.

91-DAY FEEDING STUDIES IN RATS AND MICE

METHODS

Effects of multiple exposures of p-chlorophenyl methyl sulfide, sulfoxide, and sulfone were investigated further in Fischer 344 rats and $B_6C_3F_1$ mice. Results of the 28-day range finding studies demonstrated that administration of the test compounds via the diet could produce toxicity without severely reducing food intake, thereby eliminating the necessity of a 91-day gavage study. Compounds were administered via the diet for 28, 63, or 91 days at dosage levels determined from the results of the 28-day range finding study. Three dosage levels were chosen with the following objectives:

(1) High toxic dose - a dose which induces substantial toxic changes but does not cause death in more than 20% of the animals after 30 days of treatment.

(2) Low toxic dose - a dose which induces toxic changes but does not cause death in any animals after 30 days of treatment (Intermediate, between 1 and 3).

(3) Maximum tolerated dose - a dose which induces no clinical or pathological abnormalities and not more than 10% decrease in weight gain relative to controls.

Actual dosage levels used in the 91-day feeding studies are given in Table 32.

Forty males and forty females of each species were treated at each dosage level for each compound and terminated according to the schedule given in Table 33. Due to a delay in receiving sulfoxide from the manufacturer, sulfone and sulfide studies were conducted concurrently using a common control group and sulfoxide studies were conducted later using a separate control group.

Rats weighing 100-140 g and mice weighing 17-21 g used in the studies were supplied by Charles River Breeding Laboratories. Rats and mice were individually housed in polycarbonate cages (size 19-1/2 in.

TABLE 32. DOSAGE LEVELS FOR RATS AND MICE IN THE 91-DAY FEEDING STUDIES

	Sulfide			Sulfone			Sulfoxide		
	Exposure Period[a]			Exposure Period[a]			Exposure Period[a]		
	28	63	91	28	63	91	28	63	91
Dosage Levels, ppm MICE	6000	6000	3000[b]	6000	6000	6000	5000	5000	5000
	3000	3000	1500	3000	3000	3000	3000	3000	3000
	1500	1500	750	1500	1500	1500	1500	1500	1500
	750	750				750[c]	750	750	750
Dosage Levels, ppm RATS	3000	3000	3000	3000	3000	3000	3000	3000	3000
	1500	1500	1500	1500	1500	1500	1500	1500	1500
	750	750	750	750	750	750	750	750	750

(a) Days.

(b) The 91-day sacrifice group was started later after 100% lethality had been attained at the 6000 ppm level; therefore, the 6000 ppm level was omitted from this group.

(c) A 750 ppm level was added to the 91-day sacrifice group in order to provide a level which was anticipated to be a maximum tolerated dose. It was not, however, considered necessary to place mice on study at 28- and 63-day sacrifice intervals at this level.

TABLE 33. SACRIFICE SCHEDULE FOR RODENTS

	Exposure Period (da)	Recovery Period (da)	Number of Treatment Animals Necropsied	Number of Control Animals Necropsied	Total Number Necropsied
Sulfide and Sulfone	28	0	72	20	92
	28	14	72	0	72
	63	0	72	20	92
	63	14	72	0	72
	91	0	120	28	148
	91	14	72	0	72
Sulfoxide[a]	28	0	36	12	48
	28	14	36	0	36
	63	0	36	12	48
	63	14	36	0	36
	91	0	60	10	70
	91	14	36	10	46
				Total	832

832 per species x 2 species = 1664 rodents

(a) One-half of the control rats and mice in the 91-day exposure groups were sacrificed after 91 days and the other one-half were sacrificed following an additional 14 days.

long x 10 in. wide x 6 in. high) with stainless steel barred lids. Water was supplied via glass bottles with rubber stoppers placed on the top of each cage. Purina Rat Chow(R) (meal form) was supplied ad libitum (Ralston-Purina Company, St. Louis, Missouri). Constant temperature and humidity and light/dark cycle of 12 hours was maintained. The animals were placed under quarantine for a period of at least 1 week and were observed daily for any abnormalities.

After being released from quarantine, the animals were housed under the conditions stated above. Each rodent was given a temporary identification number and was weighed using a Mettler PT15 scale. Animals with outlying weights were excluded from the study. All rodents were randomized according to weight classification by the technique described under General Methods (Appendix A). Animals were permanently identified by ear tags and cage cards.

During the week beginning on June 25, 1978, the housing configuration for rats was changed from individual to multiple housing (2-3 rats per cage, depending on group size). The time of this change corresponded to week 9 of the sulfone and sulfide studies and to week 4 of the sulfoxide study. Similarly, an attempt was made to house mice five per cage beginning with week 4 of the sulfoxide study. As a result of excessive aggressive behavior following 24 hours of multiple housing, mice were returned to individual housing. Mice in the sulfide and sulfone studies were individually housed throughout the study.

Diet Preparation

Diets were prepared by mixing the appropriate quantity of sulfide, sulfone, or sulfoxide (dissolved or suspended in corn oil) with Purina Rat Chow (meal form) (Ralston-Purina Company, St. Louis, Missouri) in one of three stainless steel, twin-shell blenders (Patterson-Kelley Company, East Stroudsburg, Pennsylvania) for 30-45 minutes, depending on the blender used. Diets were prepared in quantities sufficient for 1 week and stored at 5°C in clearly labeled, sealed polyethylene containers.

The blender chosen for mixing was a function of the quantity of diet prepared: (1) a 4-quart blender equipped with an intensifier bar for amounts less than 2000 grams (30 minute mixing time); (2) a 16-quart blender equipped with an intensifier bar for amounts from 2000 to 8000 grams (30 minute mixing time); and (3) a 75-quart blender for amounts in excess of 8000 grams (45 minute mixing time).

A PT320 Mettler balance weighing to 0.001 g was used to weigh the compounds and smaller quantities of corn oil (incorporated into the feed as 1% of total weight to improve mixing (Mazola Corn Oil, Best Foods, Englewood Cliffs, New Jersey). Feed and larger quantities of corn oil were weighed on a PT15 Mettler balance weighing to 1 g. Both balances were checked for proper calibration with known weights at the start of each day that diets were prepared.

Prior to use, sulfone and sulfoxide solids at room temperature were ground to fine powder with a ceramic mortar and pestle and then forced through a 60-mesh sieve. Sulfide, a liquid at room temperature, was allowed to thaw prior to use. The appropriate amount of compound was added to a beaker containing corn oil and mixed for approximately 5 minutes until homogeneous. This mixture was then transferred to a blender containing approximately one-half of the total amount of dry feed needed for the test diet. The beaker was dry-washed several times with feed; washings and remaining feed were added to the blender and mixed for the prescribed period of time. Recovery weights were recorded and grab samples were obtained from each batch of feed prepared.

Compound concentrations in the test diets were confirmed by gas chromatographic analysis of randomly selected grab samples. One-gram samples of the test diets were extracted three times with 2 ml of chloroform (final volume was adjusted to 10 ml). The turbid extract was filtered and injected directly into a gas chromatograph (Varian Aerograph Model 2100) under the following conditions: 200 C injection port temperature, 240 C detector temperature, 150-230 C column temperature (4 C/minute), and a 6' x 1/4 in. I.D. glass column packed with 10% FFAB on 80-100 mesh Chromosorb W-AW.

Standard feed samples were prepared by spiking feed with known quantities of compound dissolved in chloroform. The chloroform was allowed to evaporate and the standard feed samples were extracted and analyzed in the same manner as the test diets. Results of the gas chromatographic analysis of test diet and standard feed samples were compared to results of standard solutions of sulfone, sulfide, or sulfoxide in chloroform.

Observations

Animals were observed twice each day throughout the study for signs of clinical abnormalities. Technicians were required to make an entry on a daily observation form (Appendix A, Exhibit A) for each observation made. If an animal was noted to be abnormal, a description of the abnormality was recorded on a clinical observation form (Appendix A, Exhibit A). A daily entry was made on this form as long as the animal remained abnormal.

Individual body weights and food consumptions during the weeks of individual housing were obtained at weekly intervals using a Mettler Model PS15 balance (Mettler Instrument Corporation, Princeton, New Jersey). Weekly cage food consumptions were obtained during the weeks of group housing. Each feeder cup was filled with diet to a predetermined gross weight. Food consumption was obtained by subtracting the gross feeder weight at the end of the week from the initial gross weight. Any addition of feed during the week was added to this value. These data were recorded on weekly weight gain and food consumption forms (Appendix A, Exhibit E).

Electrocardiographic and ophthalmic examinations were conducted in rats prior to the onset of the study and at the end of the 91-day exposure period. Six males and six females from the control and highest dosage group for each compound were examined. Rats from the lower dosage groups and recovery groups were not evaluated since no treatment-related abnormalities were observed at the 3000 ppm dosage group in animals terminated immediately following the last day of compound administration.

Electrocardiograms were obtained using a system of three simultaneous leads to provide orthogonal lead electrocardiograms. The three leads were I, aV_f, and V_{10}. An Irex$^{(R)}$ amplifier and optical recording system with frequency response better than required for the high heart rate of rats were used. A small analogue computer was used to provide a spatial magnitude ECG, integral ECG, and spatial velocity ECG. These data were recorded at the time of examination. Information obtained included heart rate and rhythms, conduction velocities, potential degree of hypertrophy, and visible changes in waveform indicative of regional anomalies. Interpretation of the recorded information was made by a consulting cardiologist.

Prior to ophthalmic examination, pupils were dilated by instillation of one drop of mydriacil (Alcon Laboratories) into the conjunctival sac of each eye. Approximately one-half hour after instillation of the drops, the rat eyes were examined using a Welch Allyn Direct Ophthalmoscope for fundoscopic examination and an American Optical Slit-Lamp Biomicroscope for examination of the iris, lens, cornea, and conjunctivae (the anterior chamber). All rat eye exams were done by a trained and experienced veterinary ophthalmologist.

Hematology and Clinical Chemistry

Hematology and clinical chemistry parameters were evaluated for six male and six female rats from the high dose and control groups from 28- and 63-day exposures and for all rats in all groups from the 91-day exposure. If there were changes in parameters from treated groups as compared to controls in the 91-day studies, those parameters were also evaluated in the recovery group.

On the day of necropsy, blood specimens for hematological determinations were collected via the orbital plexus. Prior to sacrifice, each animal was anesthetized with an intraperitoneal injection of sodium pentobarbital and exsanguinated by cardiac puncture. Serum samples were obtained for clinical chemistry determinations by allowing the specimens of cardiac blood to coagulate. The following determinations were made:

Hematology

Erythrocyte count (RBC)
Hemoglobin (HGB)
Hematocrit (HCT)
Leukocyte count (WBC)
Differential leukocyte counts
(a) Neutrophil (SEG)
(b) Lymphocyte (LYMPH)
(c) Monocyte (MONO)
(d) Basophil (BASO)
(e) Eosinophil (EOS)

Calculated:
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)

Clinical Chemistry

Blood urea nitrogen (BUN)
Bilirubin
Serum glutamate oxalate transaminase (SGOT)
Serum glutamate pyruvate transaminase (SGPT)
Alkaline phosphatase
Sodium
Potassium
Calcium.

Necropsy and Histopathology

Prior to sacrifice, rodents were anesthetized with sodium pentobarbital or CO_2 and then exsanguinated. Detailed necropsies were performed on all animals. Organ weights were obtained from all rats and mice in the 91-day exposure groups including those animals terminated following a 2-week recovery period. Tissues removed for histology and organs weighed at necropsy are listed in Table 34. Tissue specimens were preserved in a 10% solution of neutral buffered formalin, except for the eyes which were fixed in glutaraldehyde. A 15:1 fixative to tissue ratio was maintained for all tissues. Tissues that were examined microscopically were embedded in paraffin, sectioned at 6 microns, and stained with hematoxylin and eosin for histological examination. All tissues from high dose and control animals terminated at 28- and 91-days were examined.

TABLE 34. TISSUES REMOVED FOR HISTOPATHOLOGY

Gross lesions	Tissue masses or suspect tumors and regional lymph nodes
Skin	Colon
Mandibular lymph node	Cecum
Mammary gland	Rectum
Salivary gland	Mesenteric lymph node
Thigh muscle	Liver(a)
Sciatic nerve	Gallbladder
Sternebrae, vertebrae, or femur, including marrow	Pancreas
Costochrondrial junction, rib	Spleen
Thymus	Kidneys(a)
Larynx	Adrenals(a)
Trachea	Urinary bladder
Lungs and bronchi	Seminal vesicles(a)
Heart(a)	Prostate
Thyroids(b)	Testicle with epididymis(a)
Parathyroids	Ovaries
Esophagus	Uterus(a)
Stomach	Brain(a)
Duodenum	Pituitary(b)
Jejunum	Spinal cord
Ileum	Eyes

(a) Organ weighed for rats and mice in the 91-day exposure groups (including recovery animals).

(b) Organ weighed for rats only in the 91-day exposure groups (including recovery).

If lesions were present in these animals, the tissues which contained the lesions were examined at succeedingly lower doses until a level was reached wherein the lesion was not apparent. The only tissues examined from recovery groups were those in which lesions were present in the 28- or 91-day sacrifice groups.

Statistical Analyses

Statistical analyses for rodent parameters were performed separately for each sex on weekly body weights and food consumptions, on total body weight gain, total food consumption and total feed efficiency, and on organ weights, hematology, and clinical chemistry determinations made at the end of the study period.

Differences between the treatment groups and the controls were determined by the analysis of variance (ANOVA) except for organ weights where the ancillary information provided by final body weight was used in the analysis of covariance. This method removes the effects of differences in body weight on the organ weight analysis, improving the detection of treatment effects on organ weights (Shirley, 1977a).

For parameters for which Bartlett's test was significant, the ANOVA was replaced by the nonparametric Kruskal-Wallis test (Kruskal and Wallis, 1952), and the control versus treatment differences were determined by a nonparametric analog of William's test given by Shirley (1977b).

The ANOVA's and covariance analyses were performed on Battelle's CDC 6500/CYBER 173 computer systems using the SPSS (Nie et al., 1975) and BMD (Dixon, 1975) statistical packages, respectively. A custom FORTRAN program was written to perform the Williams/Shirley tests and to produce summary statistics and significance tables.

RESULTS

Rats

Rats treated with 3000 ppm sulfone, sulfide, or sulfoxide were emaciated throughout most of the exposure period as a result of marked reductions in food and water intake. Rats at the 3000 ppm exposure levels were observed eating bedding during the study. Ocular and nasal discharges, symptoms that were observed following acute exposure, were also observed in rats treated with 3000 ppm sulfone, sulfide, or sulfoxide. Rough hair coat and inactivity were common observations made for animals at the high dose levels.

Mortality data for rats in the 91-day feeding study are presented in Table 35. One male rat in the 750 ppm sulfone group died spontaneously during the fourth week of treatment. Death was preceded by a large and sudden reduction in food consumption and body weight. Dehydration and pulmonary congestion were noted at necropsy; cause of death was not considered to be treatment related. No other spontaneous deaths were noted in rats treated with sulfone. All rats receiving sulfide survived 91 days of treatment. Six male rats receiving 3000 ppm sulfoxide died prior to their scheduled sacrifice; most male rats at this level were severely emaciated throughout the study. These deaths were attributed to compound administration.

Mean body weights and total body weight gains for rats in the 91-day feeding study are presented in Tables 36-38. Statistically significant decrements were observed for weekly body weights of male rats receiving sulfone, sulfide, or sulfoxide at dosage levels of 750, 1500, and 3000 ppm throughout the 13-week period. The same results were obtained for female rats with the exception of the 750 ppm sulfone group which did not show a statistically significant reduction in body weight at week 4. Total body

TABLE 35. SPONTANEOUS DEATHS OF RATS IN THE 91-DAY FEEDING STUDY[a]

	Control[b]		Sulfone						Sulfide						Sulfoxide					
	0.0 ppm		3000 ppm		1500 ppm		750 ppm		3000 ppm		1500 ppm		750 ppm		3000 ppm		1500 ppm		750 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Week	24[c]	24	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Cumulative	0/24	0/24	0/16	0/16	0/16	0/16	1/16	0/16	0/16	0/16	0/16	0/16	0/16	0/16	6/16	0/16	0/16	0/16	0/16	0/16

(a) Values do not include deaths occurring in rats in the 28- and 63-day interim sacrifices. No deaths occurred during the 14-day recovery period following 91 days of treatment.

(b) Data from the separate control groups were combined.

(c) Number of animals at risk.

TABLE 36. BODY WEIGHTS OF RATS IN THE 91-DAY FEEDING STUDY-SULFONE[a]

Dose, ppm	Initial	Week 4	Week 8	Week 13	Total Body Weight Gain
Male					
Control	137.8 ± 4.85	243.4 ± 9.37	291.4 ± 8.23	319.1 ± 14.03	181.29 ± 12.28
750	138.7 ± 6.96	202.0 ± 31.80[b]	261.3 ± 14.47[b]	284.4 ± 17.01[b]	145.78 ± 14.91[b]
1500	139.1 ± 7.84	195.2 ± 11.59[b]	228.0 ± 15.63[b]	249.0 ± 19.74[b]	109.90 ± 18.22[b]
3000	138.1 ± 5.69	163.2 ± 6.75[b]	186.0 ± 7.89[b]	209.0 ± 14.09[b]	70.90 ± 10.68[b]
Female					
Control	120.1 ± 4.34	152.2 ± 4.82	170.3 ± 7.57	181.2 ± 8.14	61.1 ± 9.73
750	120.2 ± 4.59	147.5 ± 2.64	159.6 ± 5.52[b]	164.9 ± 7.46[b]	44.7 ± 8.77[b]
1500	119.8 ± 6.05	138.5 ± 12.49[b]	153.0 ± 6.43[b]	156.7 ± 9.55[b]	36.9 ± 9.12[b]
3000	120.8 ± 5.75	125.9 ± 6.40[b]	139.1 ± 7.92[b]	146.3 ± 7.69[b]	25.5 ± 9.05[b]

(a) Values are group means ± standard deviation expressed in grams.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 37. BODY WEIGHTS OF RATS IN THE 91-DAY FEEDING STUDY-SULFIDE[a]

Dose, ppm	Initial	Week 4	Week 8	Week 13	Total Body Weight Gain
Male					
Control	137.8 ± 4.85	243.4 ± 9.37	291.4 ± 8.23	319.1 ± 14.03	181.3 ± 12.28
750	136.8 ± 7.73	216.4 ± 10.41[b]	257.3 ± 10.23[b]	284.9 ± 20.60[b]	148.1 ± 21.12[b]
1500	138.4 ± 8.28	176.6 ± 30.73[b]	224.7 ± 13.17[b]	248.2 ± 10.09[b]	109.8 ± 9.62[b]
3000	139.2 ± 9.73	152.9 ± 11.96[b]	174.0 ± 10.47[b]	192.3 ± 12.43[b]	53.1 ± 9.87[b]
Female					
Control	120.1 ± 4.34	152.2 ± 4.82	170.3 ± 7.56	181.2 ± 8.14	61.1 ± 9.73
750	121.1 ± 6.40	145.3 ± 7.06[b]	156.6 ± 10.13[b]	161.3 ± 10.81[b]	40.2 ± 5.33[b]
1500	120.3 ± 6.48	136.7 ± 6.63[b]	152.8 ± 8.53[b]	156.3 ± 9.67[b]	36.0 ± 7.75[b]
3000	120.3 ± 4.83	121.4 ± 6.04[b]	134.5 ± 5.17[b]	139.3 ± 10.02[b]	19.0 ± 8.87[b]

(a) Values are group means ± standard deviation expressed in grams.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 38. BODY WEIGHTS OF RATS IN THE 91-DAY FEEDING STUDY-SULFOXIDE[a]

Dose, ppm	Initial	Week 4	Week 8	Week 13	Total Body Weight Gain
Male					
Control	121.2 ± 4.37	241.7 ± 6.46	285.7 ± 6.95	332.8 ± 7.71	211.6 ± 8.19
750	122.2 ± 5.81	204.0 ± 16.26[b]	234.4 ± 22.75[b]	281.0 ± 21.82[b]	158.8 ± 18.71[b]
1500	123.5 ± 5.48	187.0 ± 7.36[b]	208.9 ± 9.72[b]	247.9 ± 11.89[b]	124.4 ± 14.83[b]
3000	124.8 ± 4.92	143.7 ± 19.45[b]	146.2 ± 45.98[b]	201.7 ± 9.89[b]	75.6 ± 8.04[b]
Female					
Control	104.6 ± 5.56	150.9 ± 6.69	165.1 ± 8.31	182.5 ± 7.31	77.9 ± 4.70
750	103.0 ± 9.76	143.4 ± 9.74[b]	154.0 ± 7.69[b]	163.5 ± 8.29[b]	60.5 ± 6.52[b]
1500	106.1 ± 4.38	137.4 ± 4.72[b]	149.1 ± 8.12[b]	159.9 ± 6.97[b]	53.8 ± 4.83[b]
3000	107.1 ± 5.25	118.8 ± 6.97[b]	125.8 ± 7.87[b]	138.2 ± 9.77[b]	31.1 ± 7.48[b]

(a) Values are group means ± standard deviation expressed in grams.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

weight gains for 13 weeks were significantly decreased in a dose-related manner for both male and female rats receiving sulfone, sulfide, or sulfoxide.

Dose-related decrements in body weight throughout 13 weeks of exposure are clearly demonstrated in Figures 1-6. Growth of rats previously receiving 3000 ppm sulfide, sulfone, or sulfoxide showed a rapid increase during the 14-day recovery period on control diet. Growth of rats at lower levels of exposure showed less rapid increases during recovery. Despite increased growth during recovery, body weights of rats in all treatment groups were still significantly less than control rats after 15 weeks (Figures 5 and 6).

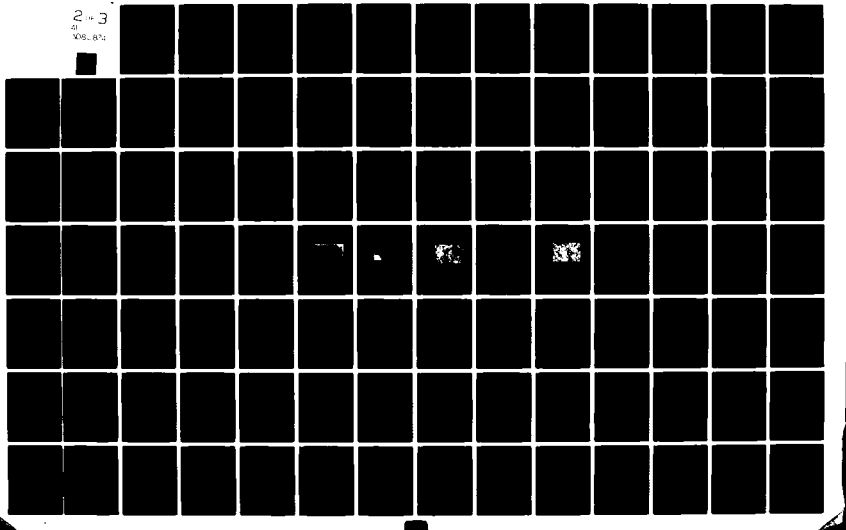
Weekly food consumptions, total food consumptions, and total feed efficiencies for rats in the 91-day feeding study are presented in Tables 39-41.

Statistically significant dose-related decrements in food consumption during week 4 were observed for both male and female rats treated with sulfone. This trend was not apparent during week 8 and week 13 when, in some groups, food consumption of treated animals exceeded that of control animals. Total food consumption for 91 days was decreased in a dose-related manner for both male and female rats receiving sulfone. Total feed efficiency also decreased in a dose-related manner.

Dose-related decrements in food consumption during week 4 and week 8 were observed for male and female rats treated with sulfide; the 1500 and 3000 ppm levels produced statistically significant differences at both 4 and 8 weeks while 750 ppm levels were statistically different from control at 8 weeks in female rats only. At 13 weeks, only the female rats receiving 3000 ppm sulfide showed significantly lower food consumption. Total food consumption was significantly less for male and female rats in the 1500 and 3000 ppm groups. Total feed efficiency decreased in a dose-related manner.

Dose-related decreases in food consumption were found for male and female sulfoxide-treated rats during week 4. During week 8, significant decrements were observed only for male and female rats in the 3000 ppm group. At 91 days, only female rats in the 3000 ppm group

AD-A082 824 BATTELLE COLUMBUS LABS OH F/G 6/20
MAMMALIAN TOXICOLOGICAL EVALUATION OF P-CHLOROPHENYL METHYL SUL--ETC(U)
JUL 79 D C THAKE, D MAYS, P LEBER, D METCALF DAMD17-77-C-7038
UNCLASSIFIED NL



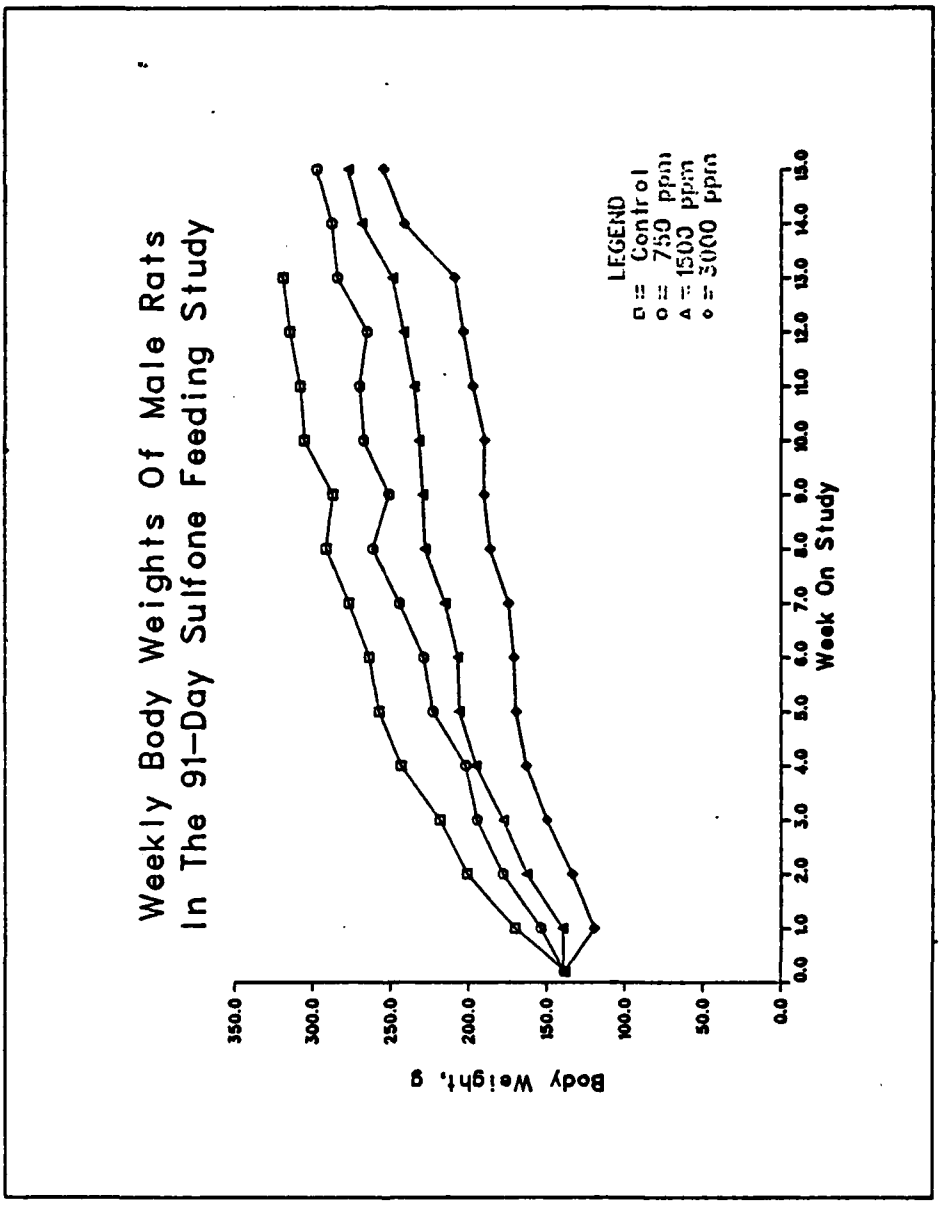

Weekly Body Weights Of Male Rats
In The 91-Day Sulfone Feeding Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm
Body Weight, g
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
Week On Study

FIGURE 1

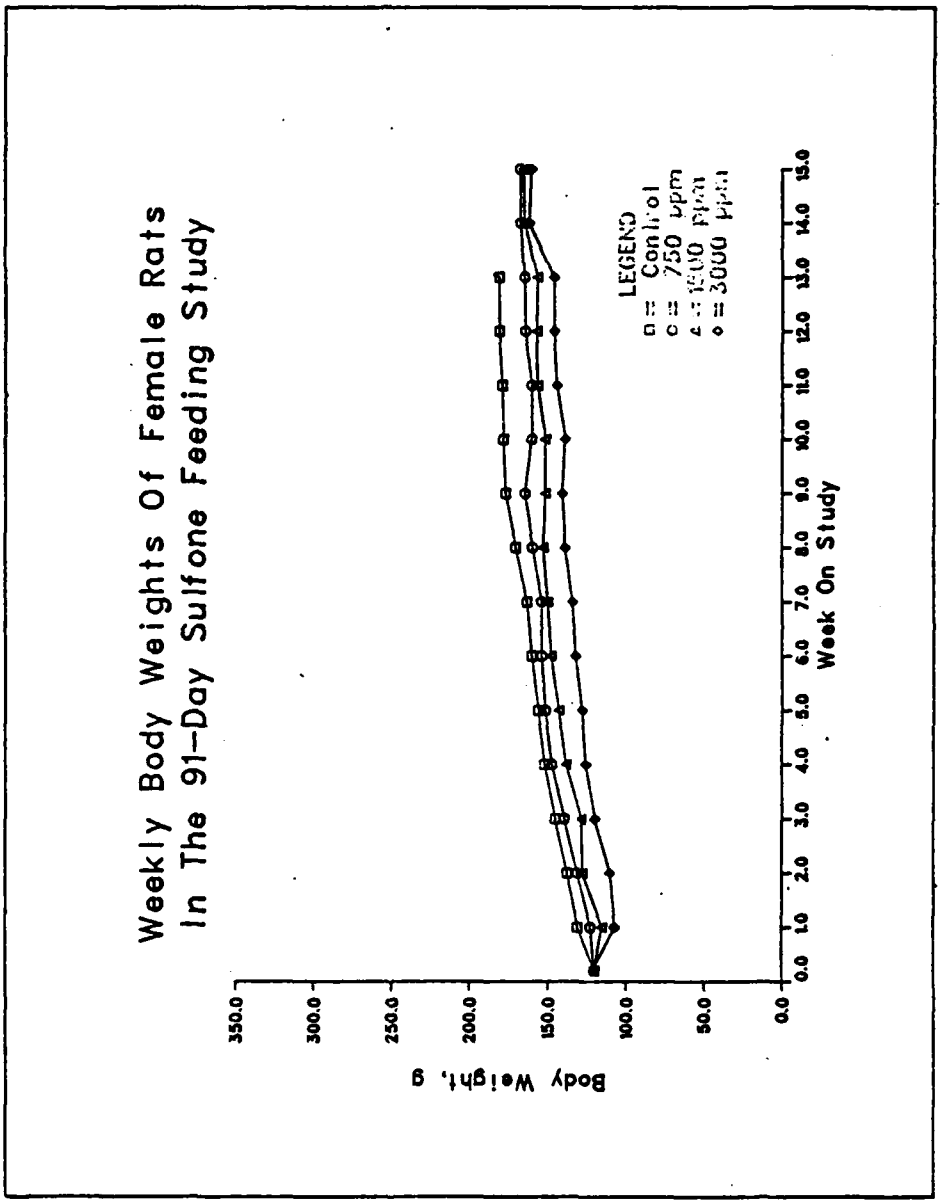

Weekly Body Weights Of Female Rats
In The 91-Day Sulfone Feeding Study
Body Weight, g
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
Week On Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm

FIGURE 2

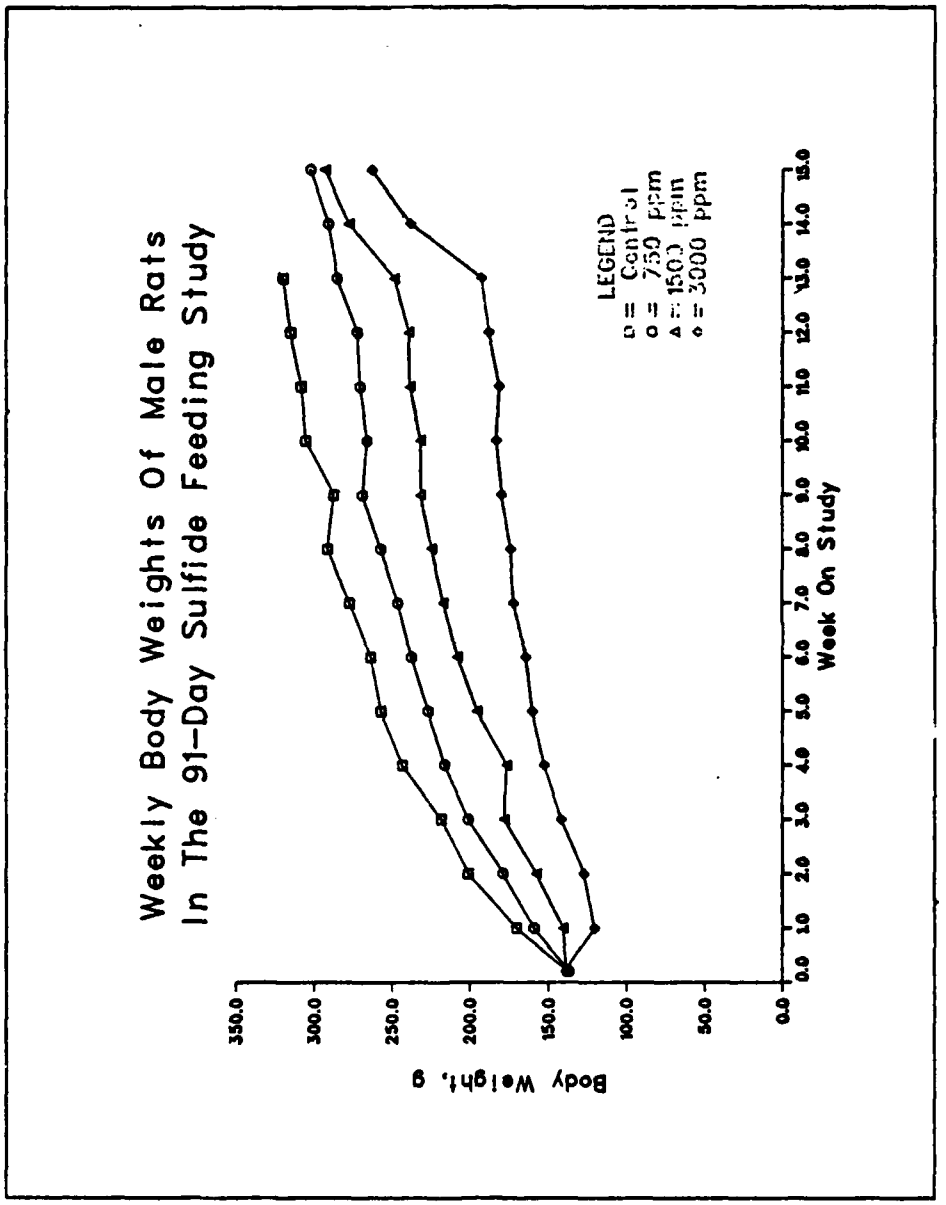

Weekly Body Weights Of Male Rats
In The 91-Day Sulfide Feeding Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm
Body Weight, g
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
Week On Study

FIGURE 3

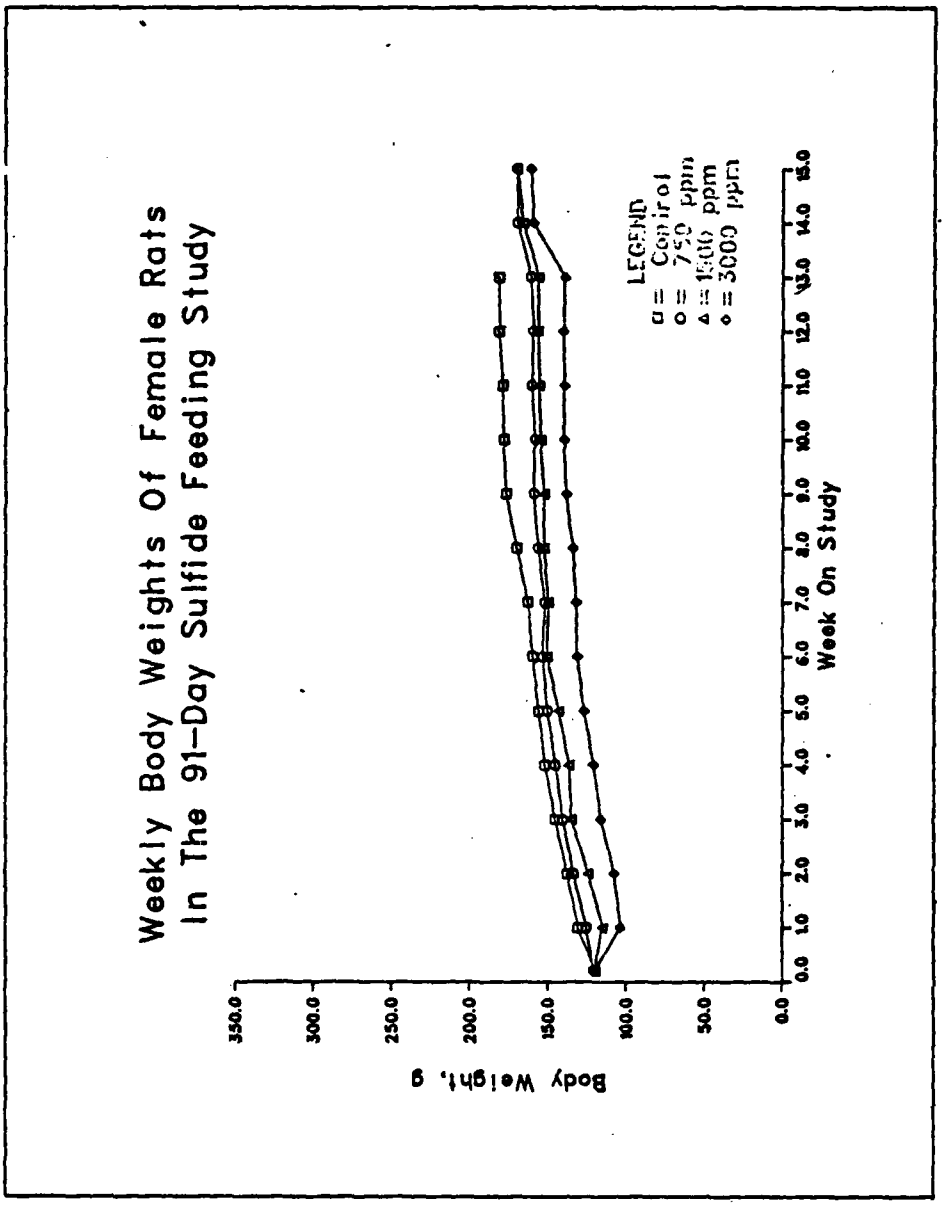

Weekly Body Weights Of Female Rats
In The 91-Day Sulfide Feeding Study
Body Weight, g
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
Week On Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm

FIGURE 4

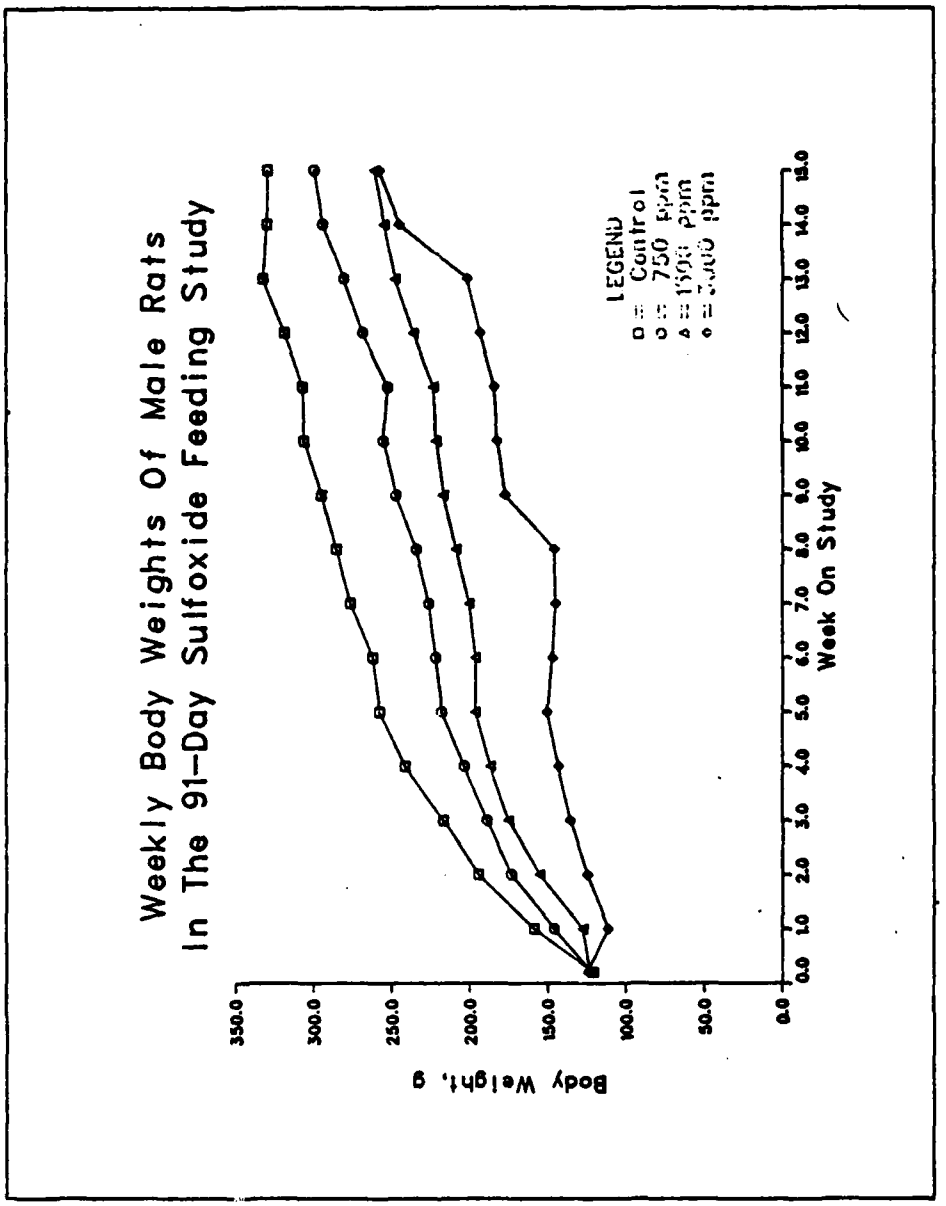

Weekly Body Weights Of Male Rats
In The 91-Day Sulfoxide Feeding Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm
Body Weight, g
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
Week On Study

FIGURE 5

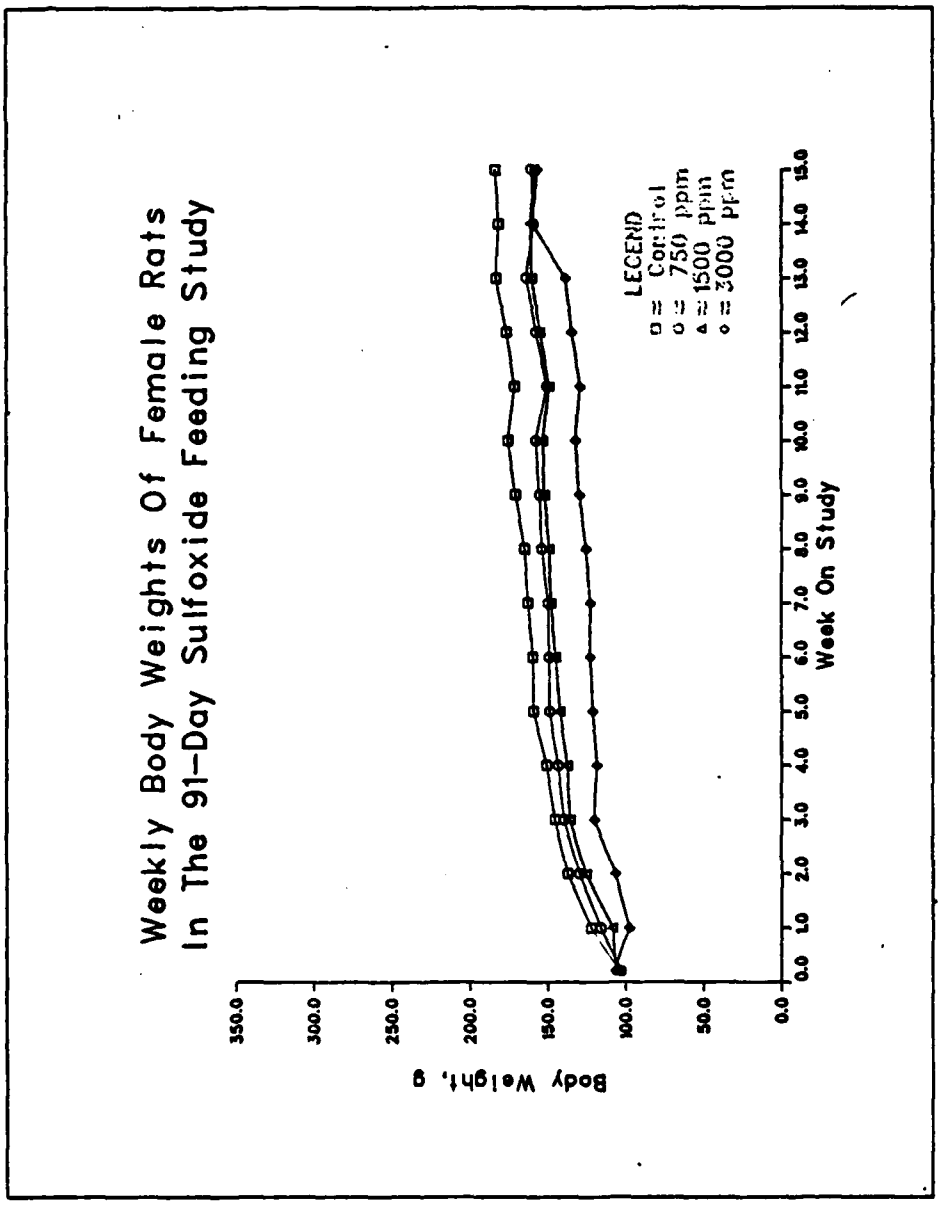

Weekly Body Weights Of Female Rats
In The 91-Day Sulfoxide Feeding Study
Body Weight, g
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
Week On Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm

FIGURE 6

TABLE 39. WEEKLY AND TOTAL FOOD CONSUMPTIONS AND TOTAL FEED EFFICIENCIES OF RATS IN THE 91-DAY FEEDING STUDY-SULFONE[a]

Dose, ppm	Week 4	Week 8	Week 13	Total Food Consumption	Total Feed Efficiency
Male					
Control	119.0 ± 8.30	116.1 ± 6.79	112.1 ± 16.44	1541.3 ± 40.38	11.8 ± 0.27
750	106.1 ± 16.27[b]	120.6 ± 7.42	129.6 ± 9.43	1503.9 ± 42.74	9.7 ± 0.42
1500	101.5 ± 13.91[b]	106.8 ± 6.83[b]	118.2 ± 8.17	1419.0 ± 81.50	7.8 ± 0.58
3000	83.1 ± 9.23[b]	91.1 ± 8.53[b]	117.8 ± 24.20	1276.6 ± 183.91	5.6 ± 0.73
Female					
Control	82.7 ± 4.89	80.8 ± 7.64	80.9 ± 6.23	1062.9 ± 54.20	5.8 ± 0.60
750	79.5 ± 5.95	70.3 ± 9.60[b]	75.7 ± 4.83	1046.2 ± 33.70	4.3 ± 0.58
1500	74.4 ± 9.89[b]	77.6 ± 11.37[b]	91.1 ± 21.07	1114.5 ± 76.50	5.3 ± 0.22
3000	60.7 ± 5.77[b]	62.8 ± 5.69[b]	81.7 ± 27.00	898.9 ± 81.50	2.8 ± 1.07

(a) Values are group means ± standard deviation. Food consumptions are expressed in grams and feed efficiencies are expressed in percent.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 40. WEEKLY AND TOTAL FOOD CONSUMPTIONS AND TOTAL FEED EFFICIENCIES OF RATS IN THE 91-DAY FEEDING STUDY-SULFIDE[a]

Dose, ppm	Week 4	Week 8	Week 13	Total Food Consumption	Total Feed Efficiency
Male					
Control	119.0 ± 8.30	116.1 ± 6.79	112.1 ± 16.44	1541.3 ± 40.38	11.8 ± 0.27
750	117.6 ± 6.47	112.8 ± 7.74	147.0 ± 19.13	1560.0 ± 50.31	9.8 ± 0.74
1500	96.6 ± 15.46[b]	102.1 ± 8.13[b]	113.7 ± 10.27	1352.1 ± 39.78	7.7 ± 0.61
3000	80.4 ± 10.28[b]	82.1 ± 6.87[b]	94.5 ± 3.34	1089.6 ± 10.27	4.2 ± 0.52
Female					
Control	82.7 ± 4.89	80.8 ± 7.64	80.9 ± 6.23	1062.9 ± 54.16	5.8 ± 0.60
750	80.3 ± 6.52	74.2 ± 6.56[b]	79.3 ± 3.81	1024.1 ± 63.52	3.8 ± 0.24
1500	73.0 ± 10.80[b]	70.3 ± 4.85[b]	77.2 ± 3.53	996.4 ± 31.50	3.2 ± 0.40
3000	63.5 ± 5.93[b]	59.1 ± 3.84[b]	67.4 ± 3.88[b]	828.2 ± 27.77	2.1 ± 0.37

(a) Values are groups means ± standard deviation. Food consumptions are expressed in grams and feed efficiencies are expressed in percent.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 41. WEEKLY AND TOTAL FOOD CONSUMPTIONS AND TOTAL FEED EFFICIENCIES OF RATS IN THE 91-DAY FEEDING STUDY-SULFOXIDE[a]

Dose, ppm	Week 4	Week 8	Week 13	Total Food Consumption	Total Feed Efficiency
Male					
Control	152.3 ± 4.69	111.5 ± 7.17	122.0 ± 3.47	1588.5 ± 35.07	13.3 ± 0.32
750	125.1 ± 8.47[b]	110.4 ± 10.71	120.7 ± 17.08	1542.7 ± 104.86	10.3 ± 0.27
1500	113.5 ± 8.82[b]	99.1 ± 6.68	117.2 ± 3.64	1384.5 ± 16.29	9.0 ± 0.81
3000	78.6 ± 12.04[b]	83.2 ± 17.93[b]	103.0 ± 4.02	1155.0 ± 22.63	6.5 ± 0.98
Female					
Control	94.8 ± 6.52	99.9 ± 5.33	82.9 ± 8.23	1124.5 ± 82.43	6.9 ± 0.45
750	87.3 ± 7.84	93.9 ± 9.79	81.1 ± 10.00	1059.0 ± 81.71	5.7 ± 0.41
1500	77.2 ± 4.09[b]	90.4 ± 7.78	78.0 ± 1.65	1008.2 ± 38.21[b]	5.3 ± 0.52
3000	66.6 ± 11.94[b]	67.5 ± 9.50[b]	66.4 ± 4.33[b]	840.0 ± 47.61[b]	3.7 ± 0.39

(a) Values are group means ± standard deviation. Food consumptions are expressed in grams and feed efficiencies are expressed in percent.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

consumed significantly less feed than did control rats. Total feed consumption showed dose-related decreases in both male and female rats. Total feed efficiency decreased in a dose-related manner.

Dose-related inhibitions in food consumption observed during weeks 1-4 for rats receiving sulfone, sulfide, or sulfoxide are clearly illustrated in Figures 7-12. Also, reversal of this inhibition in food consumption for rats at the 750 or 1500 ppm levels during the final two-thirds of the exposure period can be seen. Food consumption of rats in the high dose groups exceeded that of control rats by as much as 50% during week 14 but returned to control values during week 15 (Figures 11 and 12).

Body weight and food consumption data showed similar relationships with all three compounds. During the initial weeks of treatment, food consumptions were depressed in a dose-related manner; poor palatibility of the diet or toxicity could have produced this result. Decrements in body weights were also noted during this period.

Poor palatibility of the diet or toxicity could hav contributed to the decreased food consumption observed during the initial stages of treatment. Decrements in body weights observed during this period correlate very well with decrements in food consumption. During final weeks of treatment, food consumption of treated animals, in many cases, equaled or exceeded control values. However, growth during the later weeks did not show a similar trend.

During the final weeks of treatment, food consumption of treated animals showed substantial gains as compared to controls and, in many instances, food consumption of animals in the 750 and 1500 ppm groups equaled or exceeded control values. Body weights of treated animals did not show a similar increase and growth appeared to be retarded throughout the 91-day exposure period.

Mean hematology values determined from blood specimens taken immediately prior to sacrifice following 91 days of treatment are presented in Table 42. Most hematology values for treated animals were statistically significantly different from control animals. Differences observed for hemoglobin, hematocrit, and erythrocyte counts for male and female rats

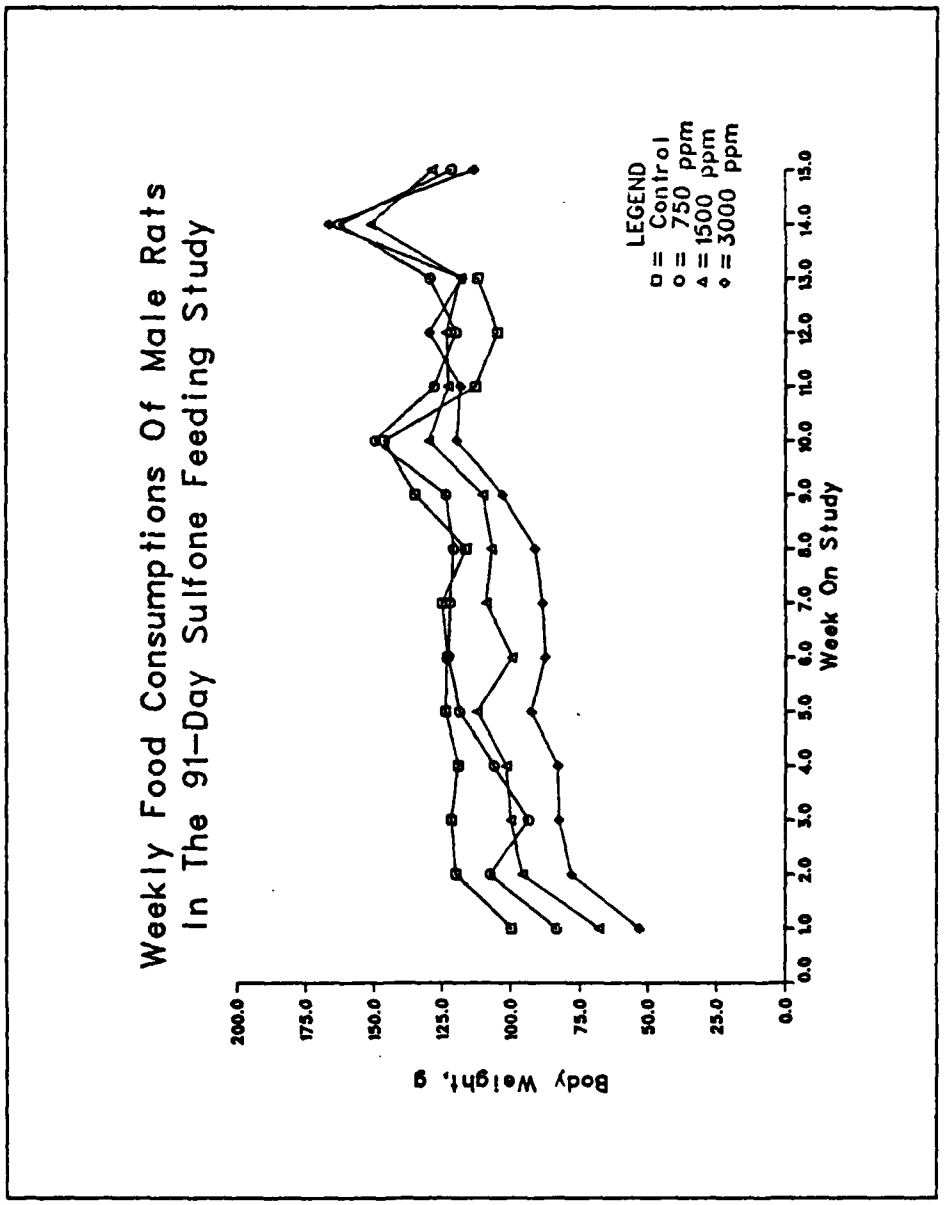

Weekly Food Consumptions Of Male Rats
In The 91-Day Sulfone Feeding Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm
Body Weight, g
200.0
175.0
150.0
125.0
100.0
75.0
50.0
25.0
0.0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
Week On Study

FIGURE 7

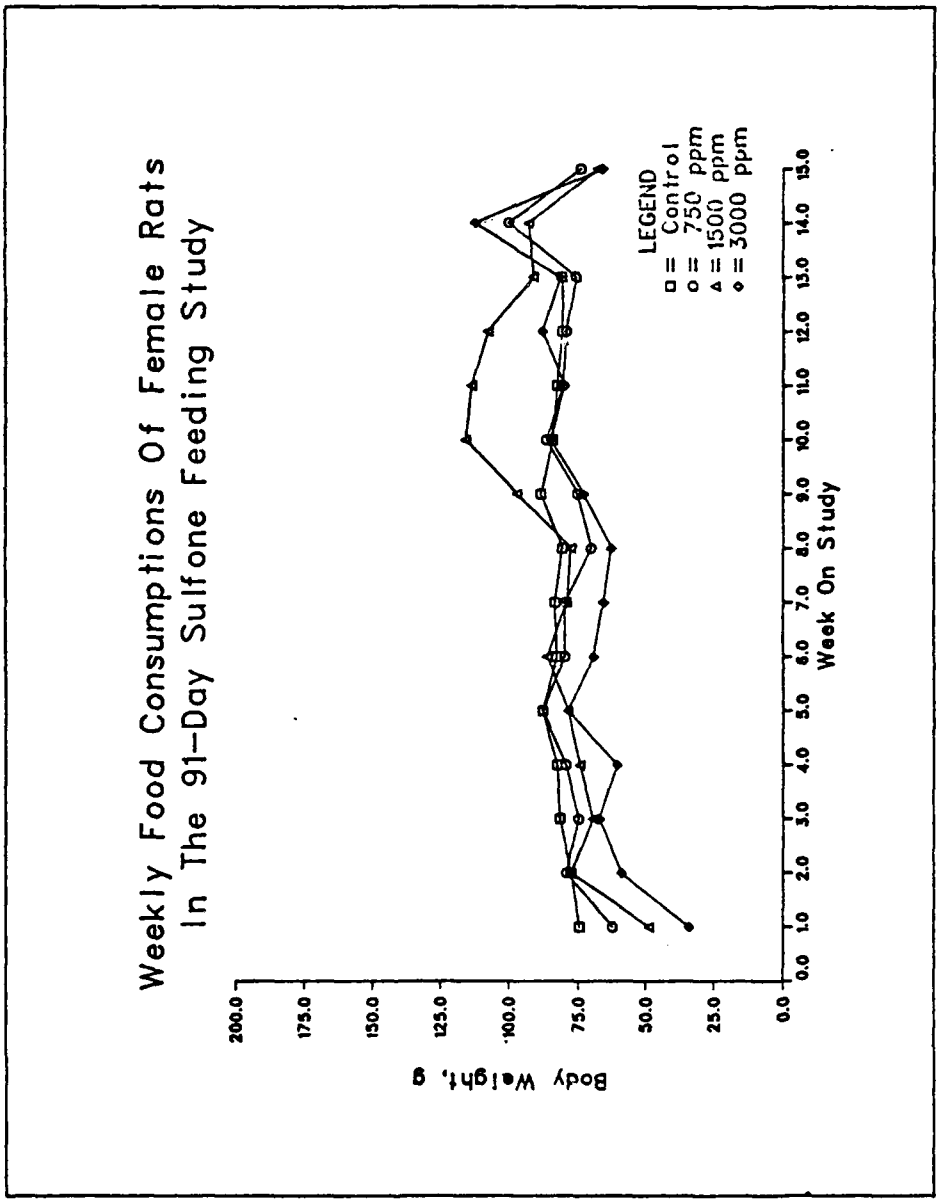

Weekly Food Consumptions Of Female Rats
In The 91-Day Sulfone Feeding Study
Body Weight, g
200.0
175.0
150.0
125.0
100.0
75.0
50.0
25.0
0.0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
Week On Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm

FIGURE 8

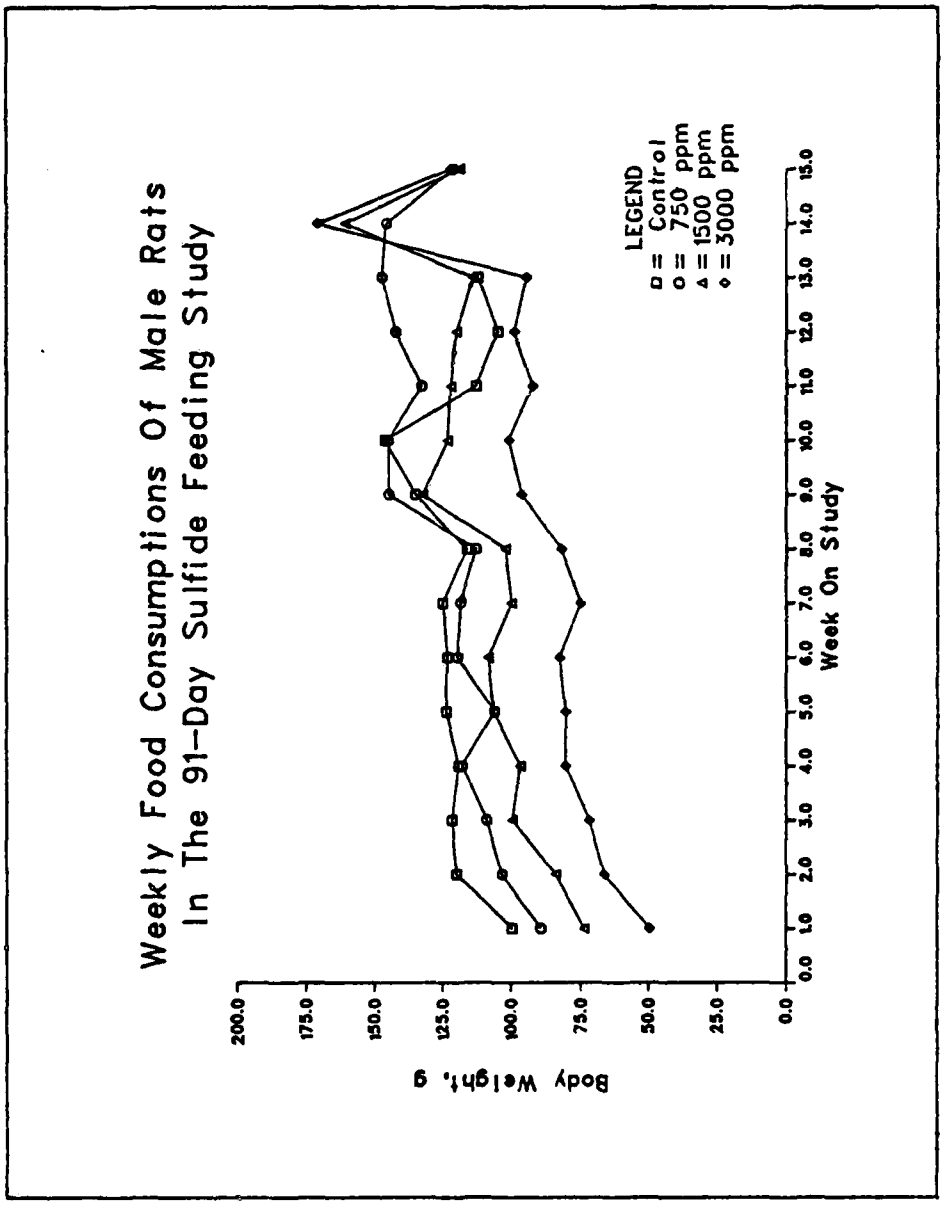

Weekly Food Consumptions Of Male Rats
In The 91-Day Sulfide Feeding Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm
Body Weight, g
200.0
175.0
150.0
125.0
100.0
75.0
50.0
25.0
0.0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
Week On Study

FIGURE 9

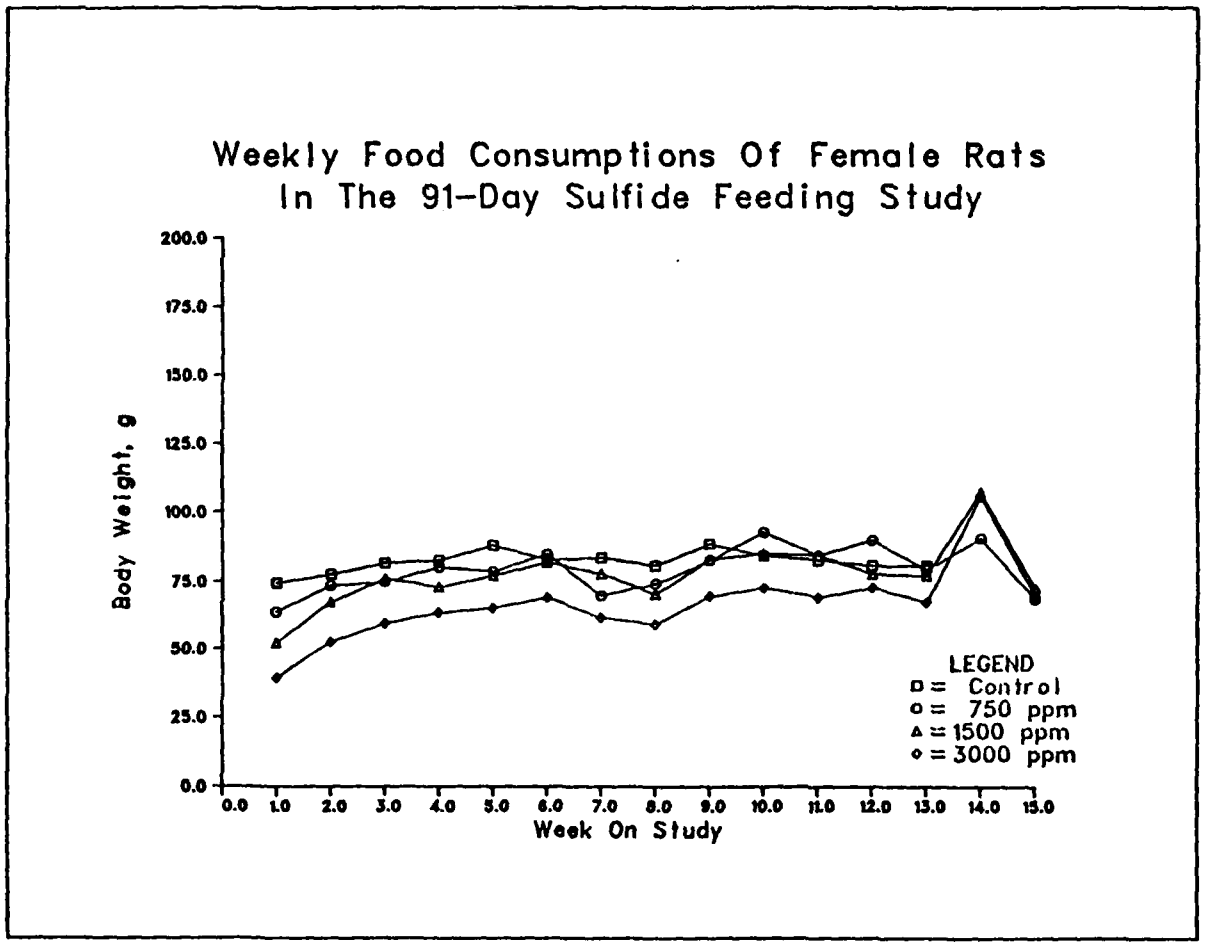

Weekly Food Consumptions Of Female Rats
In The 91-Day Sulfide Feeding Study
Body Weight, g
200.0
175.0
150.0
125.0
100.0
75.0
50.0
25.0
0.0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
Week On Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm

FIGURE 10

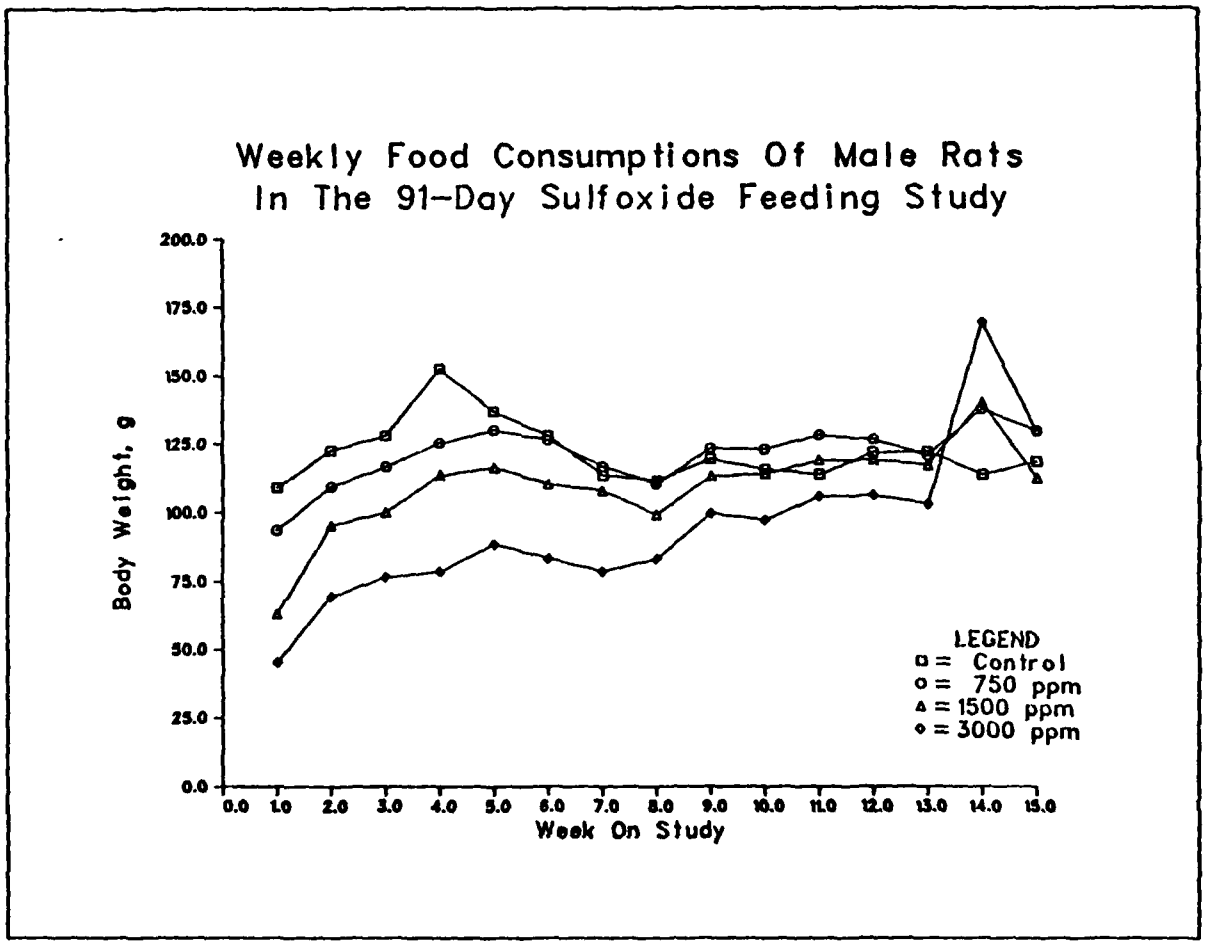

Weekly Food Consumptions Of Male Rats
In The 91-Day Sulfoxide Feeding Study
Body Weight, g
200.0
175.0
150.0
125.0
100.0
75.0
50.0
25.0
0.0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
Week On Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm

FIGURE 11

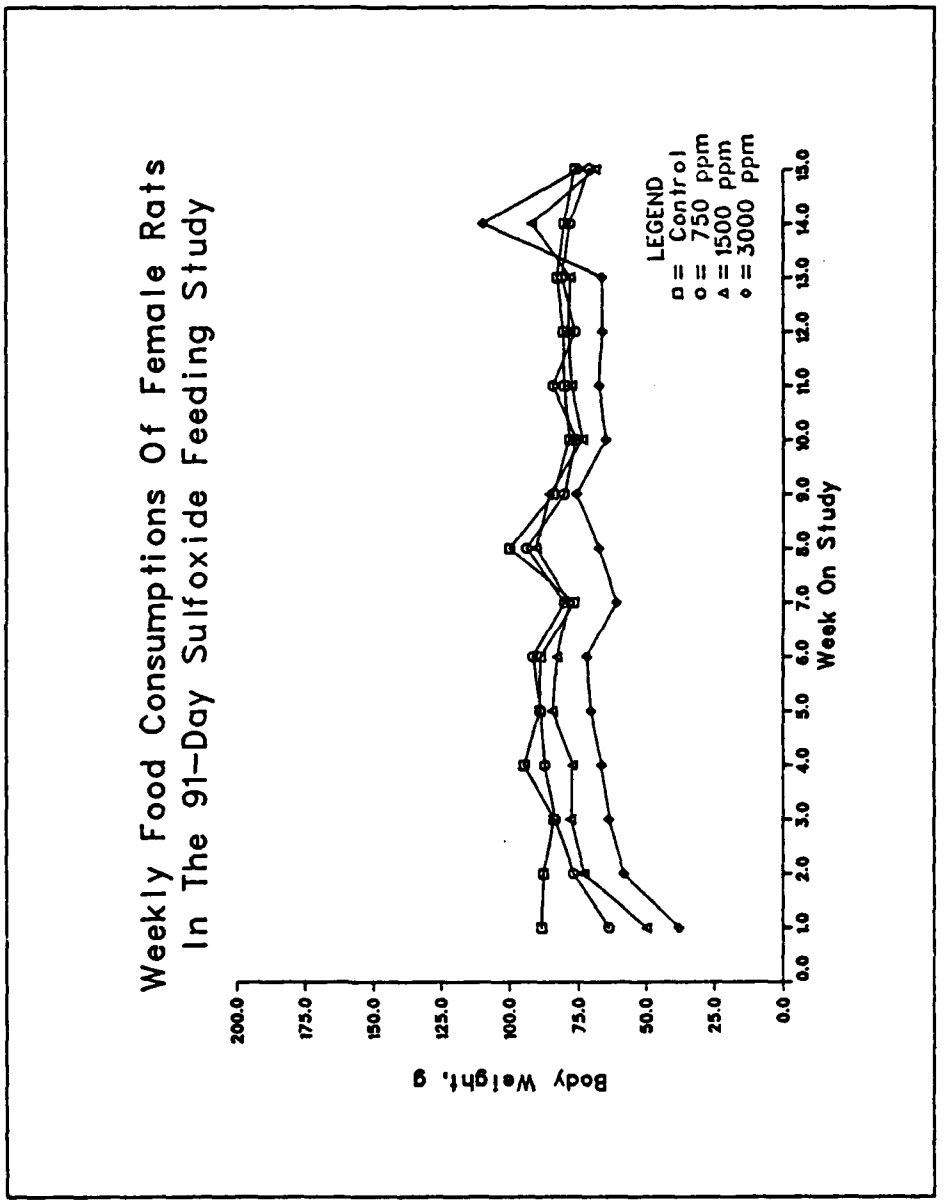

Weekly Food Consumptions Of Female Rats
In The 91-Day Sulfoxide Feeding Study
Body Weight, g
200.0
175.0
150.0
125.0
100.0
75.0
50.0
25.0
0.0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0
Week On Study
LEGEND
□ = Control
○ = 750 ppm
△ = 1500 ppm
◇ = 3000 ppm

FIGURE 12

TABLE 42. HEMATOLOGY DETERMINATIONS FOR RATS IN THE 91-DAY FEEDING STUDY-SULFONE, SULFIDE, AND SULFOXIDE[a]

Parameter	Sulfone			Sulfide			Control	Sulfoxide			Control
	3000 ppm	1500 ppm	750 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm
Male											
HGB (g %)	15.9	15.3	15.1	15.6[b]	15.3[b]	15.2[b]	16.7	15.8	15.9	14.7	16.8
HCT (%)	42.8[b]	40.9[b]	40.6[b]	41.8[b]	41.4[b]	41.2[b]	47.1	45.3[b]	46.8[b]	43.3[b]	49.0
WBC ($10^3/mm^3$)	6.68	8.05	7.49	6.49	7.01	8.21	7.32	6.07	6.08	7.09	6.05
RBC ($10^6/mm^3$)	8.46[b]	8.34[b]	8.39[b]	8.35[b]	8.47[b]	8.37[b]	9.48	8.62[b]	9.06[b]	8.21[b]	9.48
MCV (μm^3)	51.3	49.4	49.0	50.6[b]	49.5[b]	49.8[b]	50.9	53.1[b]	52.2[b]	53.4[b]	52.6
MCH (pg)	18.8[b]	18.3[b]	18.0[b]	18.7[b]	18.1[b]	18.2[b]	17.7	18.3[b]	17.6[b]	17.9[b]	17.7
MCHC (g %)	36.7[b]	37.1[b]	36.8[b]	37.0[b]	36.6[b]	36.5[b]	34.7	34.5[b]	33.7[b]	33.6[b]	33.7
Female											
HGB (g %)	16.1[b]	15.9[b]	15.8[b]	16.0[b]	15.4[b]	16.0[b]	16.8	15.3[b]	15.5[b]	15.2[b]	16.3
HCT (%)	44.1[b]	42.8[b]	42.4[b]	44.9[b]	41.7[b]	43.2[b]	48.1	43.3[b]	45.2[b]	44.6[b]	46.8
WBC ($10^3/mm^3$)	7.65	8.11	8.11	7.25	7.32	7.96	7.69	5.06	6.54	6.12	5.01
RBC ($10^6/mm^3$)	8.55[b]	8.33[b]	8.31[b]	8.65[b]	8.17[b]	8.36[b]	8.88	8.42	8.46	8.48	8.64
MCV (μm^3)	52.1[b]	51.6[b]	51.6[b]	52.4[b]	51.6[b]	52.0[b]	54.7	51.9[b]	53.9	53.2	54.9
MCH (pg)	18.8	19.1	19.0	18.5	18.8	19.1	18.9	18.2[b]	18.4[b]	18.0[b]	18.9
MCHC (g %)	36.1	37.0[b]	36.8[b]	35.3[b]	36.4[b]	36.7[b]	34.5	35.1[b]	34.1[b]	33.8[b]	34.4

(a) Values are group means.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

receiving sulfone, sulfide, or sulfoxide were considered to be biologically significant. No microscopic changes in H&E-stained bone marrow sections of treated rats were observed that correlated with these differences. The statistically significant differences found for mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were small and were not considered biologically significant.

Clinical chemistry values determined from blood specimens taken immediately prior to sacrifice after 91 days of treatment are given in Table 43. Specimens with evidence of hemolysis were excluded from the calculations.

Blood urea nitrogen values (BUN) were significantly elevated in male rats receiving sulfone at all treatment levels; values were increased for male rats receiving 750 and 1500 ppm sulfide or sulfoxide but not enough to be statistically significant using William's test. No significant difference in BUN values was observed for female rats.

Serum glutamic oxaloacatic transaminase (SGOT) values were statistically significantly lower for male rats in the 3000, 1500, and 750 ppm groups treated with sulfide or sulfoxide. SGOT values for sulfone-treated male rats were also depressed but not significantly. The SGPT value for females given 3000 ppm of sulfoxide were also significantly decreased below control levels. Although remarkable changes were observed microscopically in livers of treated rats, no evidence was found to support decreases in serum SGOT or SGPT values.

Alkaline phosphatase (AP) levels for both male and female rats receiving sulfone, sulfide, or sulfoxide at all possible levels were significantly less than control values. The magnitude of the changes were small. Serum potassium and calcium levels were markedly elevated for both male and female rats treated with sulfone, sulfide, or sulfoxide. Serum sodium was decreased at higher dosage levels of sulfoxide and generally similar to control levels in animals given sulfone and sulfide with the exception of females treated with sulfide in which sodium values were higher than those of controls. This generally resulted in substantially decreased sodium/potassium ratios which was most profound in males and females from the sulfone group and females from the sulfoxide group.

TABLE 43. CLINICAL CHEMISTRY DETERMINATIONS FOR RATS IN THE 91-DAY FEEDING STUDY WITHOUT RECOVERY-SULFONE, SULFIDE, SULFOXIDE[a]

Parameter	Sulfone			Sulfide			Control	Sulfoxide			Control
	3000 ppm	1500 ppm	750 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm
Male											
BUN	24.0[b]	25.6[b]	25.4[b]	19.2	23.0	25.3	19.4	22.8	26.7	27.2	24.9
SGOT	87.5	85.9	80.9	56.0[b]	71.5[b]	54.0	114.6	75.8[b]	97.4[b]	97.5[b]	216.1[c]
SGPT	49.5	49.4	43.1	33.8	40.3	29.6	35.2	44.2	54.1	49.7	102.1[c]
Alk Phos	130.0[b]	128.6[b]	124.2[b]	143.8[b]	124.0[b]	131.5[b]	159.5	132.2[b]	123.2[b]	139.2[b]	157.1
Sodium	143.6	142.9	143.0	142.4	140.9	141.5	141.9	144.4[b]	148.6	148.8	147.4
Potassium	6.14[b]	5.80[b]	5.33[b]	5.48[b]	5.40[b]	5.58[b]	4.41	5.76[b]	5.70[b]	5.77[b]	4.97
Calcium	5.58	5.35	5.77	6.08[b]	6.34[b]	6.26[b]	5.50	5.84[b]	5.80[b]	5.71[b]	5.40
Female											
BUN	24.8	21.3	20.8	24.0	20.1	20.1	19.8	22.1	20.0	23.1	25.1
SGOT	82.6	146.3	86.0	124.3[b]	57.0	61.1	130.0	78.5[b]	100.2[b]	132.9	167.9
SGPT	36.7	62.9	34.2	81.3	30.1	32.3	36.6	30.8[b]	43.0	54.8	60.9
Alk Phos	112.7[b]	103.1[b]	97.3[b]	138.1[b]	86.9[b]	99.5	146.8	131.1[b]	91.9[b]	100.8[b]	169.0
Sodium	143.9[b]	143.1[b]	142.7[b]	147.1[b]	149.6[b]	141.4	141.8	144.9[b]	144.6[b]	147.7[b]	151.9
Potassium	6.28[b]	6.38[b]	5.38[b]	5.41[b]	6.66[b]	5.89[b]	4.43	6.14	5.94	6.39	5.56
Calcium	6.23[b]	5.61[b]	5.67	6.06[b]	6.30[b]	6.18[b]	5.22	6.04[b]	5.99[b]	5.57	5.41

(a) Values are group means.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

(c) SGOT and SGPT values for two rats in this group were extremely elevated and considered to be spurious. Mean values for SGOT and SGPT excluding these data are 146.0 and 57.6 I.U., respectively.

Hematology and clinical chemistry determinations performed on specimens collected from rats following 91 days of exposure with no recovery period and following 91 days of exposure with a 14-day recovery period are given in Tables 44-47. Ten (five per sex) sulfoxide control rats were sacrificed at 91 days and 10 were sacrificed at 105 days. Hematology and clinical chemistry values determined for sulfoxide control rats at 91 and 105 days were statistically equivalent using Student's t-test and thereby pooled for subsequent comparisons with recovery and nonrecovery treatment rats. Control rats for sulfone and sulfide were sacrificed at 91 days. Values determined at 91 days for sulfide and sulfone rats were used for comparisons with recovery and nonrecovery treatment rats. BUN was the only clinical chemistry determination made for sulfide and sulfone recovery rats.

Following 14 days on control diet, values for hemoglobin, hematocrit, and erythrocyte counts of most treatment groups remained below control group values; female rats showed a slightly greater improvement following recovery for these parameters.

Serum glutamic oxaloacetic transaminase levels in rats remained lower than control values following the 14-day recovery period (Table 47). However, the differences were not statistically significant. Also, alkaline phosphatase activity did not return to control levels following recovery. The increases observed in potassium and calcium levels following 91 days of treatment were abolished after 14 days of recovery. Sodium levels remained significantly lower for females in the 3000 ppm sulfoxide treatment group; all other groups were not significantly different from controls following recovery. Sodium:potassium ratios were near normal for sulfoxide rats following recovery.

Mean organ weights of rats that were sacrificed immediately following 91 days of exposure (without recovery) are presented in Table 48. Decrements in heart, pituitary, thyroid, brain, spleen, testicle, and ovary weights in treated rats were approximately proportional to decrements in body weights; organ weight responses were similar for all compounds although dose relationships were not always apparent.

TABLE 44. COMPARISON OF HEMATOLOGY AND CLINICAL CHEMISTRY DETERMINATIONS FOR RATS IN THE 91-DAY FEEDING STUDY WITH RECOVERY AND WITHOUT RECOVERY-SULFONE[a]

Parameter	Sulfone Without Recovery			Sulfone With Recovery			Control
	3000 ppm	1500 ppm	750 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm
Male							
HGB (g %)	15.9	15.3	15.1	15.5[b]	15.6[b]	15.4[b]	16.7
HCT (%)	42.8[b]	40.9[b]	40.6[b]	43.6[b]	42.5[b]	41.8[b]	47.1
WBC (10^3/mm^3)	6.68	8.05	7.49	7.88[b]	8.32[b]	8.32[b]	7.32
RBC (10^6/mm^3)	8.46[b]	8.34[b]	8.39[b]	8.69[b]	8.64[b]	8.68[b]	9.48
MCV (μm^3)	51.3	49.4	49.0	50.7[b]	49.5[b]	48.8[b]	50.9
MCH (pg)	18.8[b]	18.3[b]	18.0[b]	17.8	18.0	17.7	17.6
MCHC (g %)	36.7[b]	37.1[b]	36.8[b]	35.2[b]	36.4[b]	36.3[b]	34.7
BUN (mg/dl)	24.0[b]	25.6[b]	25.4[b]	21.0	20.7	22.5	19.7
Female							
HGB (g %)	16.1[b]	15.9[b]	15.8[b]	16.0	16.7	16.3	16.8
HCT	44.1[b]	42.8[b]	42.4[b]	44.5[b]	45.2[b]	43.7[b]	48.1
WBC (10^3/mm^3)	7.65	8.11	8.11	7.20	8.12	7.35	7.69
RBC (10^6/mm^3)	8.55[b]	8.33[b]	8.31[b]	8.69	9.11	8.81	8.88
MCV (μm^3)	52.1[b]	51.6[b]	51.6[b]	51.7[b]	50.0[b]	50.0[b]	54.7
MCH (pg)	18.8	19.1	19.0	18.4[b]	18.4[b]	18.5[b]	18.9
MCHC (g %)	36.1	37.0[b]	36.8[b]	35.7[b]	36.7[b]	37.0[b]	34.5
BUN (mg/dl)	24.8	21.3	20.8	17.5	18.0	19.0	20.4

(a) Values are group means.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 45. COMPARISON OF HEMATOLOGY AND CLINICAL CHEMISTRY DETERMINATIONS FOR RATS IN THE 91-DAY FEEDING STUDY WITH RECOVERY AND WITHOUT RECOVERY-SULFIDE[a]

Parameter	Sulfide Without Recovery			Sulfide With Recovery			Control
	3000 ppm	1500 ppm	750 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm
Male							
HGB (g %)	15.6[b]	15.3[b]	15.2[b]	15.3	15.3	15.0	16.7
HCT (%)	41.8[b]	41.4[b]	41.2[b]	45.7	45.8	42.2	47.1
WBC ($10^3/mm^3$)	6.49	7.01	8.21	6.77	6.65	7.62	7.32
RBC ($10^6/mm^3$)	8.35[b]	8.47[b]	8.37[b]	8.58	8.75	8.74	9.48
MCV (μm^3)	50.6[b]	49.5[b]	49.8[b]	53.7[b]	52.8	48.8	50.9
MCH (pg)	18.7[b]	18.1[b]	18.2[b]	17.9	17.4	17.1	17.7
MCHC (g %)	37.0[b]	36.6[b]	36.5[b]	33.3	33.0	35.0	34.7
BUN (mg/dl)	19.2[b]	23.0[b]	25.3[b]	20.8[b]	23.6[b]	23.0[b]	19.4
Female							
HGB (g %)	16.0[b]	15.4[b]	16.0[b]	15.2[b]	15.9[b]	16.1[b]	16.8
HCT (%)	44.9[b]	41.7[b]	43.2[b]	45.7[b]	47.0[b]	45.0[b]	48.1
WBC ($10^3/mm^3$)	7.25	7.32	7.96	6.60	6.17	7.07	7.69
RBC ($10^6/mm^3$)	8.65[b]	8.17[b]	8.36[b]	8.51	8.89	9.03	8.88
MCV (μm^3)	52.4[b]	51.6[b]	52.0[b]	54.0[b]	53.3[b]	50.7[b]	54.7
MCH (pg)	18.5	18.8	19.1	17.9[b]	17.9[b]	17.8[b]	18.9
MCHC (g %)	35.5[b]	36.4[b]	36.7[b]	33.2[b]	33.5[b]	35.1	34.5
BUN (mg/dl)	24.0	20.1	20.1	18.8	18.8	20.6	19.8

(a) Values are group means.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 46. COMPARISON OF HEMATOLOGY DETERMINATIONS FOR RATS IN THE 91-DAY FEEDING STUDY-SULFOXIDE[a]

Parameter	Sulfoxide Without Recovery			Sulfoxide With Recovery			Control
	3000 ppm	1500 ppm	750 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm
Male							
HGB (g %)	15.8[b]	15.9[b]	14.7[b]	15.3[b]	15.6[b]	15.2[b]	16.8
HCT (%)	45.3[b]	46.8[b]	43.3	42.0[b]	43.2[b]	42.2[b]	49.0
WBC ($10^3/mm^3$)	6.07	6.08[b]	7.09[b]	5.13	6.10	6.28	6.05
RBC ($10^6/mm^3$)	8.62[b]	9.06[b]	8.21[b]	8.47	8.78	8.62	9.48
MCV (μm^3)	53.1	52.2	53.4	50.3	49.7	49.3	52.6
MCH (pg)	18.3[b]	17.6	17.9	18.0	17.8	17.6	17.7
MCHC (g %)	34.5	33.7	33.6	35.8[b]	35.8[b]	35.7	33.7
Female							
HGB (g %)	15.3[b]	15.5[b]	15.2[b]	15.2[b]	16.2	16.6	16.3
HCT (%)	43.3[b]	45.2[b]	44.6[b]	42.8[b]	43.8[b]	45.3	46.8
WBC ($10^3/mm^3$)	5.06[b]	6.54[b]	6.12[b]	3.57	5.00	4.67	5.01
RBC ($10^6/mm^3$)	8.42	8.46	8.48	8.40[b]	8.83	9.08	8.64
MCV (μm^3)	51.9	53.9	53.2	51.7[b]	50.2[b]	50.5	54.9
MCH (pg)	18.2[b]	18.4[b]	18.0[b]	18.0[b]	18.3[b]	18.2[b]	18.9
MCHC (g %)	35.1	34.1	33.8	34.9	36.5[b]	36.1[b]	34.4

(a) Values are group means.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 47. COMPARISON OF CLINICAL CHEMISTRY DETERMINATIONS FOR RATS IN THE 91-DAY FEEDING STUDY WITH RECOVERY AND WITHOUT RECOVERY-SULFOXIDE[a]

Parameter	Sulfoxide Without Recovery			Sulfoxide With Recovery			Control
	3000 ppm	1500 ppm	750 ppm	3000 ppm	1500 ppm	750 ppm	
Male							
BUN	22.8	26.7	27.2	18.7[b]	22.2	23.0	24.9
SGOT	75.8[b]	97.4[b]	97.5[b]	104.3	117.3	119.5	216.1[c]
SGPT	44.2	54.1	49.7	46.0	60.0	60.0	102.1[c]
Alk Phos	132.3[b]	123.2[b]	139.2[b]	137.6[b]	108.0[b]	141.5[b]	157.1
Sodium	144.4[b]	148.6	148.8	147.7	148.5	145.8	147.4
Potassium	5.76[b]	5.70[b]	5.77[b]	5.03	5.37	5.35	4.97
Calcium	5.84[b]	5.80[b]	5.71[b]	5.30	5.05	5.50	5.40
Female							
BUN	22.1	20.0	23.1	19.3[b]	18.7[b]	21.8[b]	25.1
SGOT	78.5[b]	100.2[b]	132.9	118.8	146.5	158.0	167.9
SGPT	30.8[b]	43.0	54.8	34.8[b]	51.7	45.0	60.9
Alk Phos	131.1[b]	91.9[b]	100.8[b]	103.8[b]	102.7[b]	121.5[b]	169.0
Sodium	144.9[b]	144.6[b]	147.7[b]	146.2[b]	150.5	150.8	151.9
Potassium	6.14	5.94	6.39	5.15	5.33	5.25	5.56
Calcium	6.04[b]	5.99[b]	5.57	5.15	5.23	5.50	5.41

(a) Values are group means.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

(c) SGOT and SGPT values for two rats in this group were extremely elevated and considered to be spurious. Mean values for SGOT and SGPT excluding these data are 146.0 and 57.6 I.U., respectively.

TABLE 48. ORGAN WEIGHTS (g) FOR RATS IN THE 91-DAY FEEDING STUDY WITHOUT RECOVERY-SULFONE, SULFIDE, AND SULFOXIDE (GROUP MEANS)

Organ	Sulfone			Sulfide			Control	Sulfoxide			Control
	3000 ppm	1500 ppm	750 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm
Male											
Heart	0.748(a)	0.884	0.954	0.742(a)	0.853	0.931	1.052	0.807(a)	0.838(a)	0.882(a)	1.015
Pituitary	0.006	ND(b)	ND	0.003	0.006	0.008	0.007	0.006(a)	0.006(a)	0.007(a)	0.009
Adrenal, pair	0.075(a)	0.090(a)	0.091(a)	0.079(a)	0.083(a)	0.083(a)	0.066	0.070(a)	0.083(a)	0.077(a)	0.062
Thyroid	0.020(a)	ND	ND	0.024	0.031	0.029	0.034	0.025	0.026	0.025	0.028
Brain	1.78(a)	1.81(a)	1.88(a)	1.74(a)	1.86(a)	1.90(a)	1.95	1.62(a)	1.84	1.87	1.87
Testicles	3.98	4.52	4.49	4.13(a)	4.26	4.47	4.85	3.31(a)	4.16(a)	4.40	4.45
Spleen	0.385(a)	0.489(a)	0.591(a)	0.369(a)	0.490(a)	0.588	0.637	0.297(a)	0.477(a)	0.561	0.615
Liver	17.77(a)	20.12(a)	21.39(a)	16.97(a)	19.89(a)	21.80(a)	10.90	15.90(a)	20.32(a)	21.30(a)	11.37
Right kidney	1.19(a)	1.38(a)	1.44(a)	1.10	1.37	1.47	1.12	1.08	1.43	1.51	1.16
Left kidney	1.20(a)	1.39(a)	1.46(a)	1.09	1.38	1.51	1.13	1.06	1.41	1.50	1.15
Seminal vesicle	0.707	ND	ND	0.889(a)	0.830(a)	0.898(a)	1.372	0.776(a)	1.088(a)	1.257	1.365
Female											
Heart	0.549(a)	0.601(a)	0.631	0.546(a)	0.606(a)	0.621(a)	0.661	0.514(a)	0.593	0.605	0.609
Pituitary	0.006(a)	0.005(a)	ND	0.006(a)	0.007(a)	0.006(a)	0.010	0.005(a)	0.006(a)	0.008(a)	0.010
Adrenal, pair	0.068	0.072	0.081	0.062(a)	0.080	0.069	0.070	0.069	0.070	0.073	0.065
Thyroid	0.022	0.025	ND	0.022	0.028	0.025	0.027	0.021	0.020	0.024	0.026
Brain	1.71	1.72	1.76	1.68(a)	1.73(a)	1.78	1.79	1.66(a)	1.74	1.74	1.79
Ovaries	0.108	0.127	0.139	0.087	0.124	0.121	0.141	0.080(a)	0.113(a)	0.117(a)	0.141
Spleen	0.309(a)	0.358(a)	0.396(a)	0.284(a)	0.384(a)	0.382(a)	0.461	0.255(a)	0.319(a)	0.348(a)	0.406
Liver	11.81(a)	12.32(a)	12.23(a)	11.84(a)	12.43(a)	12.08(a)	6.06	11.56(a)	12.38(a)	11.85(a)	5.96
Right kidney	0.719(a)	0.765(a)	0.789(a)	0.710(a)	0.771(a)	0.740(a)	0.685	0.685(a)	0.784(a)	0.763(a)	0.637
Left kidney	0.730(a)	0.770(a)	0.785(a)	0.715(a)	0.768(a)	0.742(a)	0.678	0.682(a)	0.787(a)	0.768(a)	0.636
Uterus	0.387	0.273	ND	0.251(a)	0.339(a)	0.362(a)	0.509	0.189(a)	0.289(a)	0.401(a)	0.602

(a) Significantly different from control by use of William's test or its nonparametric equivalent, $p < 0.05$.

(b) ND = no data were collected.

Adrenal weights were moderately elevated for male rats receiving sulfone, sulfide, or sulfoxide. A change of this nature is not uncommon in animals under stress induced by toxicants. Female rats did not show significant changes in adrenal weights.

Both seminal vesicle and uterus weights were markedly depressed in rats receiving sulfone, sulfide, or sulfoxide. The changes were consistent with hypoplasia observed in these organs.

Kidney weights were significantly higher, although not always statistically, in treated male and female rats receiving sulfone, sulfide, or sulfoxide. Although decreases were observed in kidney weights of male rats receiving 1500 or 750 ppm sulfide or sulfoxide, response of the high dose group did not satisfy a requirement of William's test and differences observed were not statistically significant. No lesions in kidneys were observed microscopically that would account for these changes.

Liver weights of both male and female rats treated with sulfone, sulfide, or sulfoxide were greatly increased at all dosage levels. In some groups, livers of treated animals were more than twice the size of control animals. These changes are consistent with lesions observed microscopically in rat livers and with enzyme induction as evidenced by twofold reduction in hexobarbital sleep time.

Mean organ weights of rats sacrificed after 91 days of exposure followed by 14 days of recovery are given in Table 49. Liver weights of rats in all treatment groups were significantly higher after 14 days of recovery; however, the magnitude of the differences were much less than differences observed without recovery. In most treatment groups, liver weights were less than 20% heavier than control values. This reversal is consistent with microscopic evidence for recovery animals and historical data for effects of phenobarbital-type liver enzyme inducers.

Kidney weights of treated rats remained higher following recovery, but the differences were statistically significant only for female rats in the 750, 1500, and 3000 ppm groups. Spleen weights were also significantly lower for male rats in the 1500 and 3000 ppm groups receiving sulfoxide and for female rats in most groups.

TABLE 49. ORGAN WEIGHTS (g) FOR RATS IN THE 91-DAY FEEDING STUDY WITH RECOVERY-SULFONE, SULFIDE, SULFOXIDE (GROUP MEANS)

Organ	Sulfone			Sulfide			Control	Sulfoxide			Control
	3000 ppm	1500 ppm	750 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm
Male											
Heart	0.885	0.876	0.913	0.857	0.897	0.928	1.052	0.904	0.926	0.979	1.015
Pituitary	0.007	0.007	0.008	0.009	0.008	0.009	0.007	0.007	0.010	0.009	0.009
Adrenal, pair	0.073	0.073	0.065	0.063	0.073	0.072	0.066	0.073	0.057	0.077	0.062
Thyroid	0.026	0.028	0.029	0.025	0.032	0.027	0.034	0.035	0.031	0.035	0.028
Brain	1.87[a]	1.87[a]	1.95	1.83	1.90	1.87	1.95	1.85	1.88	2.00	1.87
Testicles	4.17	4.42	4.61	3.97	4.42	4.51	4.85	4.05	4.22	4.58	4.55
Spleen	0.550	0.602	0.670	0.630	0.649	0.541	0.637	0.481[a]	0.478[a]	0.594	0.615
Liver	12.01[a]	12.77[a]	13.64[a]	12.23[a]	13.72[a]	14.15[a]	10.90	12.20[a]	11.24[a]	13.03[a]	11.37
Right kidney	1.12	1.20	1.29	1.11	1.32	1.31	1.12	1.18	1.17	1.26	1.16
Left kidney	1.12	1.22	1.27	1.11	1.35	1.36	1.13	1.11	1.16	1.26	1.15
Seminal vesicle	0.963	0.889	1.085	0.929	1.139	1.204	1.372	1.340	0.942	1.296	1.365
Female											
Heart	0.621	0.622	0.637	0.641	0.718	0.623	0.661	0.656[a]	0.649[a]	0.615	0.609
Pituitary	0.007	0.009	0.009	0.011	0.009	0.008	0.010	0.010	0.008	0.010	0.010
Adrenal, pair	0.076	0.062	0.067	0.062	0.071	0.063	0.070	0.062	0.062	0.067	0.065
Thyroid	0.023	0.019	0.025	0.032	0.022	0.021	0.027	0.022	0.021	0.029	0.026
Brain	1.70	1.75	1.76	1.73	1.80	1.79	1.79	1.71	1.74	1.78	1.79
Ovaries	0.120	0.130	0.136	0.161	0.151	0.131	0.141	0.111	0.127	0.115	0.141
Spleen	0.387[a]	0.383[a]	0.411[a]	0.396[a]	0.425	0.428	0.461	0.343[a]	0.339[a]	0.339[a]	0.406
Liver	7.07[a]	7.05[a]	7.06[a]	7.00[a]	7.11[a]	6.86[a]	6.06	6.78[a]	6.27	5.98	5.96
Right kidney	0.681	0.735	0.746	0.690	0.756	0.716	0.685	0.690[a]	0.692[a]	0.729[a]	0.637
Left kidney	0.692	0.716	0.731	0.717	0.754	0.721	0.678	0.697[a]	0.693[a]	0.712[a]	0.636
Uterus	0.451	0.511	0.594	0.443	0.490	0.525	0.509	0.415[a]	0.483[a]	0.464[a]	0.602

(a) Significantly different from control by use of William's test or its nonparametric equivalent, $p < 0.05$.

One rat from the 3000 ppm sulfone group developed a cataract with extensive neovascularization in the left eye during the study period. It was impossible to ascertain whether this lesion was related to compound ingestion although it appeared to be of a traumatic origin and not related to treatment. No other ocular lesions of any kind were observed in the rats on these compounds.

Rodent electrocardiograms revealed no significant changes from controls.

Mice

Marked decreases in body weight and food consumption prior to death were noted in mice treated with 5000 ppm sulfoxide and 6000 ppm sulfone.

Mortality data for mice in the 91-day feeding study are presented in Table 50. One male control mouse died as a result of wounds received during group housing and one female control mouse died as a result of traumatic injuries received during handling. Only one mouse (a female) survived 91 days of treatment with 6000 ppm sulfone; all other mice at this level died within the first 2 weeks of treatment. Prior to death, mice responded to treatment with a severe depression in food intake and a concomitant loss of weight (essentially the same response that was observed in rats). No other deaths were observed for mice receiving sulfone. All deaths of mice receiving 3000 ppm sulfide occurred during the first week of treatment. No deaths were observed at the 1500 or 750 ppm sulfide levels. All mice receiving 5000 ppm sulfoxide died during the first week of treatment. With the exception of mice that died from traumatic lesions, symptoms observed prior to death for mice receiving sulfoxide were the same as those observed with sulfide or sulfone.

A total of eight mice (seven treatment and one control) in the sulfoxide study died as a direct result of traumatic injuries. During a brief period of multiple housing (five per cage), mice exhibited extremely aggressive behavior. Although all mice were returned to

TABLE 50. SPONTANEOUS DEATHS OF MICE IN THE 91-DAY FEEDING STUDY[a]

	Control[b]		Sulfone								Sulfide						Sulfoxide							
	0.0 ppm		6000 ppm		3000 ppm		1500 ppm		750 ppm		3000 ppm		1500 ppm		750 ppm		5000 ppm		3000 ppm		1500 ppm		750 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Week	33[c]	34	16	16	16	16	16	15	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
1	0	0	12	10	0	0	0	0	0	0	5	12	0	0	0	0	16	16	1	4	0	0	0	0
2	0	0	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	1[d]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2[d]	0	0	1[d]	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3[d]	1[d]	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cumulative	1/33	1/34	16/16	15/16	0/16	0/16	0/16	0/15	0/16	0/16	5/16	12/16	0/16	0/16	0/16	0/16	16/16	16/16	3/16	7/16	3/16	2/16	0/16	0/16

(a) Values do not include deaths occurring in mice in the 28- and 63-day interim sacrifices. No deaths occurred during the 14-day recovery period following 91 days of treatment.

(b) Data from the separate control groups were combined.

(c) Number of animals at risk.

(d) Deaths of these animals were attributed to traumatic lesions from fighting which occurred in Week 4 during a 24-hour period of multiple housing.

individual cages within 24 hours, many lesions were severe enough to result in the deaths of several animals.

Mean body weights for mice in the 91-day feeding study are presented in Tables 51-53. Weekly body weights were depressed throughout the study for male mice receiving sulfone, sulfide, or sulfoxide at the 3000, 1500, or 750 ppm level. Total body weight gains for male mice were significantly less in all sulfone and sulfoxide treated groups. Initial body weights for sulfide mice were less than controls; however, total body weight gains were equivalent to control values. Female mice receiving sulfone showed decrements in body weights that were related to dose. Total body weight gains also were depressed in a dose-related manner, but only at the 1500 and 3000 ppm levels were differences statistically significantly different from control values. Weekly body weights and total body weight gains for female mice treated with sulfide were statistically equivalent to control mice. Female mice experienced 75% mortality in the 3000 ppm sulfide group which resulted in a small number for statistical comparisons and heavier body weights among surviving animals. The deaths of the weaker and presumably lighter mice biased the mean body weight since only stronger and healthier mice survived. Although decreases were observed in body weights of treated mice, the high dose group did not satisfy the requirements of William's test and therefore differences observed were not found to be statistically significant.

Mean weekly food consumptions for mice in the 91-day feeding study are presented in Tables 54-56. Food consumption data for mice were highly variable for all three compounds. Generally, it can be stated that treated mice consumed less feed than did control mice, as indicated by mean consumption differences. Total feed efficiencies were variable and no consistent trends were noted. It is of interest to note that at 4 weeks food consumption in treated groups was sometimes greater than that of controls, while the greatest decrease in weight gain was usually detected in the first 4 weeks.

Mean organ weights for mice in the 91-day feeding study are given in Table 57. Kidney weights were lower for male mice receiving 750, 1500, or 3000 ppm sulfide or sulfone. Spleen weights were decreased for most treated animals, as were testicle, ovary, uterus, seminal

TABLE 51. BODY WEIGHTS OF MICE IN THE 91-DAY FEEDING STUDY-SULFONE[a]

Dose, ppm	Initial	Week 4	Week 8	Week 13	Total Body Weight Gain
Male					
Control	19.9 ± 1.22	24.7 ± 1.45	27.9 ± 2.21	29.2 ± 1.50	9.1 ± 1.34
750	19.8 ± 1.17	23.0 ± 1.68[b]	24.0 ± 2.35[b]	25.4 ± 3.67[b]	5.4 ± 3.11[b]
1500	20.0 ± 1.00	23.1 ± 1.27[b]	24.4 ± 1.24[b]	25.0 ± 1.32[b]	5.0 ± 1.48[b]
3000	19.9 ± 1.01	22.5 ± 1.71[b]	24.1 ± 2.01[b]	24.8 ± 2.00[b]	4.9 ± 1.34[b]
Female					
Control	17.8 ± 0.79	21.70 ± 0.98	23.7 ± 1.15	25.1 ± 1.24	7.3 ± 1.41
750	17.7 ± 0.91	20.90 ± 1.12[b]	22.5 ± 0.99[b]	24.4 ± 1.22	6.7 ± 1.58
1500	17.6 ± 0.74	20.70 ± 1.17[b]	22.1 ± 0.83[b]	22.9 ± 0.85[b]	5.1 ± 0.85[b]
3000	17.7 ± 0.96	20.14 ± 1.14[b]	21.8 ± 1.36[b]	22.3 ± 1.37[b]	4.6 ± 1.26[b]

(a) Values are group means ± standard deviation expressed in grams.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 52. BODY WEIGHTS OF MICE IN THE 91-DAY FEEDING STUDY-SULFIDE[a]

Dose, ppm	Initial		Week 4		Week 8		Week 13		Total Body Weight Gain	
Male										
Control	19.9	1.22	24.7	1.45	27.9	2.21	29.2	1.5	9.1	1.34
750	17.9	1.11	23.7	1.01	26.0	1.47[b]	26.5	1.3[b]	8.6	1.08
1500	18.0	1.25[b]	23.6	1.46[b]	26.0	1.28[b]	27.1	1.6[b]	9.1	1.11
3000	17.8	0.88[b]	22.9	1.13[b]	25.0	1.39[b]	26.4	1.6[b]	8.6	1.17
Female										
Control	17.8	0.79	21.7	0.98	23.7	1.15	25.1	1.24	7.3	1.41
750	16.4	0.93[b]	21.3	1.35	23.4	1.40	24.4	1.65	8.0	1.25
1500	16.3	0.96[b]	21.0	1.42	22.7	1.54	24.1	1.58	7.7	0.99
3000	16.8	0.67[b]	21.8	0.32	23.7	0.90	23.8	0	7.0	0.67

(a) Values are group means ± standard deviation expressed in grams.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 53. BODY WEIGHTS OF MICE IN THE 91-DAY FEEDING STUDY-SULFOXIDE[a]

Dose, ppm	Initial	Week 4	Week 8	Week 13	Total Body Weight Gain
Male					
Control	21.1 ± 0.83	24.5 ± 1.11	27.3 ± 1.03	28.8 ± 1.00	7.6 ± 0.94
750[c]	23.0 ± 1.56	25.2 ± 1.65	26.4 ± 1.79	28.5 ± 2.64	5.6 ± 1.37
1500	21.1 ± 0.95	23.1 ± 1.63[b]	25.4 ± 1.31[b]	26.7 ± 1.58[b]	5.6 ± 0.99[b]
3000	21.2 ± 0.80	22.4 ± 1.06[b]	26.4 ± 1.15[b]	26.9 ± 1.36[b]	5.6 ± 1.80[b]
Female					
Control	18.1 ± 1.19	21.2 ± 1.33	23.4 ± 1.72	24.8 ± 2.09	6.7 ± 1.39
750[c]	19.8 ± 0.78	21.9 ± 1.32	23.4 ± 1.11	24.5 ± 1.41	4.7 ± 0.90
1500	18.0 ± 1.18	20.2 ± 1.33	21.9 ± 1.36	22.6 ± 1.51[b]	4.6 ± 0.84[b]
3000	18.2 ± 1.14	19.8 ± 1.20	22.2 ± 1.16	22.9 ± 1.62[b]	4.6 ± 1.21[b]

(a) Values are group means ± standard deviation expressed in grams.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

(c) This group was placed on study at a later date as a result of all mice dying at the 6000 ppm level. Data from these animals were not included in the William's test.

TABLE 54. WEEKLY AND TOTAL FOOD CONSUMPTIONS AND TOTAL FEED EFFICIENCIES OF MICE IN THE 91-DAY FEEDING STUDY-SULFOXIDE[a]

Dose, ppm	Week 4	Week 8	Week 13	Total Food Consumption	Total Feed Efficiency
Male					
Control	25.1 ± 3.04	26.0 ± 2.00	30.2 ± 3.56	354.7 ± 26.87	2.14 ± 0.27
750[c]	25.6 ± 4.93	30.1 ± 2.77	25.3 ± 3.53	347.9 ± 45.60	1.62 ± 0.43
1500	28.3 ± 10.94	21.6 ± 2.17	25.5 ± 4.22[b]	306.3 ± 20.84[b]	1.83 ± 0.14
3000	17.4 ± 7.16	23.5 ± 5.37	23.1 ± 6.40[b]	272.9 ± 67.81[b]	2.25 ± 0.40
Female					
Control	19.5 ± 7.38	19.1 ± 6.08	17.8 ± 9.77	264.3 ± 52.87	2.56 ± 0.47
750[c]	11.8 ± 5.31	16.0 ± 4.39	16.3 ± 3.89	206.5 ± 35.08	2.17 ± 0.14
1500	23.2 ± 6.83	22.4 ± 5.53	22.4 ± 6.67	285.3 ± 66.71	1.72 ± 0.59[b]
3000	18.5 ± 4.00	15.5 ± 4.44	12.3 ± 5.85	189.4 ± 38.15[b]	2.42 ± 0.59[b]

(a) Values are group means ± standard deviation. Food consumptions are expressed in grams and feed efficiencies are expressed in percent.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

(c) This group was placed on study at a later date as a result of all mice dying at the 6000 ppm level. Data from these animals were not included in the William's test.

TABLE 55. WEEKLY AND TOTAL FOOD CONSUMPTIONS AND TOTAL FEED EFFICIENCIES OF MICE IN THE 91-DAY FEEDING STUDY-SULFIDE[a]

Dose, ppm	Week 4	Week 8	Week 13	Total Food Consumption	Total Feed Efficiency
Male					
Control	19.7 ± 3.97	28.1 ± 7.80	28.4 ± 4.68	316.9 ± 48.22	2.93 ± 0.56
750	21.9 ± 8.73	26.1 ± 5.49[b]	22.9 ± 5.33[b]	285.4 ± 50.53	3.09 ± 0.63
1500	20.1 ± 5.22	19.7 ± 6.36[b]	20.4 ± 7.07[b]	245.3 ± 55.35[b]	3.81 ± 1.16
3000	22.9 ± 4.26	23.0 ± 5.10[b]	23.0 ± 8.39[b]	282.8 ± 33.59[b]	2.99 ± 0.53
Female					
Control	24.7 ± 7.06	26.1 ± 6.42	24.3 ± 3.54	315.3 ± 70.51	2.36 ± 0.47
750	21.9 ± 6.02	23.0 ± 8.71	20.2 ± 9.21	262.2 ± 68.73[b]	3.25 ± 1.17
1500	18.5 ± 7.08	17.8 ± 6.21[b]	16.7 ± 5.94	207.9 ± 44.28[b]	3.89 ± 1.09[b]
3000	15.3 ± 5.86	13.7 ± 6.66[b]	16.0 ± 8.00	172.3 ± 35.36[b]	4.23 ± 1.15[b]

(a) Values are group means ± standard deviation. Food consumptions are expressed in grams and feed efficiencies are expressed in percent.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 56. WEEKLY AND TOTAL FOOD CONSUMPTIONS AND TOTAL FEED EFFICIENCIES OF MICE IN THE 91-DAY FEEDING STUDY-SULFONE[a]

Dose, ppm	Week 4	Week 8	Week 13	Total Food Consumption	Total Feed Efficiency
Male					
Control	19.7 ± 3.97	28.14 ± 7.80	28.4 ± 4.68	316.9 ± 48.22	2.93 ± 0.56
750	19.4 ± 4.79	23.80 ± 4.83[b]	25.4 ± 6.05	281.0 ± 37.34	2.03 ± 1.40[b]
1500	18.5 ± 5.32	19.70 ± 4.42[b]	20.9 ± 4.98[b]	252.0 ± 56.84	2.11 ± 0.87[b]
3000	20.7 ± 9.10	23.10 ± 5.63[b]	27.3 ± 5.62[b]	289.1 ± 68.55	1.80 ± 0.73[b]
Female					
Control	24.7 ± 7.06	26.10 ± 6.42	24.3 ± 3.54	315.3 ± 70.51	2.40 ± 0.47
750	16.0 ± 4.71[b]	16.50 ± 5.66[b]	18.8 ± 5.45	211.3 ± 49.92[b]	3.40 ± 1.53
1500	17.0 ± 8.82[b]	19.80 ± 9.08[b]	22.8 ± 6.22	228.3 ± 80.62[b]	2.50 ± 1.01
3000	17.0 ± 4.12[b]	16.00 ± 4.37[b]	16.9 ± 6.84[b]	213.3 ± 30.23[b]	2.50 ± 0.65

(a) Values are group means ± standard deviation. Food consumptions are expressed in grams and feed efficiencies are expressed in percent.

(b) Significantly different from control using William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 57. ORGAN WEIGHTS (g) FOR MICE IN THE 91-DAY FEEDING STUDY-SULFONE, SULFIDE, SULFOXIDE (GROUP MEANS)

Parameter	Sulfone			Sulfide			Control	Sulfoxide			Control
	3000 ppm	1500 ppm	750 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm	3000 ppm	1500 ppm	750 ppm	0.0 ppm
Male											
Heart	0.174	0.148	0.163	0.198	0.188	0.182	0.181	0.156	0.159	0.174[b]	0.170
Adrenals, paired	0.009[a]	0.008[a]	0.008[a]	0.009	0.009	0.010	0.012	0.010[a]	0.009[a]	0.009	0.014
Brain	0.455[a]	0.468[a]	0.454[a]	0.452[a]	0.462[a]	0.468	0.481	0.458	0.461	0.458	0.465
Testicles	0.385[a]	0.424[a]	0.419[a]	0.418	0.463	0.433	0.497	0.408	0.427	0.410	0.451
Spleen	0.085	0.075	0.062	0.074	0.070	0.073	0.081	0.075[a]	0.082[a]	0.109	0.095
Liver	1.77	1.46	1.38	2.24[a]	1.77	1.68	1.92	2.61[a]	1.78	1.78	1.92
Right kidney	0.269[a]	0.278[a]	0.286[a]	0.263[a]	0.274[a]	0.293[a]	0.328	0.252	0.261	0.390	0.275
Left kidney	0.264[a]	0.271[a]	0.282[a]	0.264[a]	0.271[a]	0.291[a]	0.319	0.252	0.259	0.270	0.275
Seminal vesicle	0.244[a]	0.257[a]	0.294	0.257[a]	0.301	0.270	0.318	0.294	0.303	0.316	0.318
Female											
Heart	0.158[a]	0.122[a]	0.137[a]	0.133	0.159	0.164	0.160	0.131	0.127	0.170	0.136
Adrenals, paired	0.012	0.012	0.013	0.015	0.013	0.013	0.014	0.011	0.013	0.015	0.014
Brain	0.458[a]	0.458	0.472	0.437	0.456	0.475	0.489	0.455	0.463	0.482	0.471
Ovaries	0.032	0.034	0.042	0.034	0.033	0.037	0.039	0.032[a]	0.034[a]	0.038	0.116
Spleen	0.079[a]	0.075[a]	0.090[a]	0.089	0.094	0.094	0.102	0.074[a]	0.079[a]	0.091	0.115
Liver	1.92	1.36	1.36	2.03[a]	1.62	1.52	1.73	2.14[a]	1.52	1.45	1.57
Right kidney	0.209	0.209	0.207	0.221	0.202	0.222	0.225	0.197	0.178	0.226	0.193
Left kidney	0.199	0.212	0.210	0.219	0.198	0.217	0.221	0.207	0.171	0.219	0.215
Uterus	0.140	0.182	0.187	0.136	0.168	0.167	0.168	0.139	0.150	0.168	0.138

(a) Significantly different from control by use of William's test or its nonparametric equivalent, $p < 0.05$.

(b) This group was placed on study at a later date as a result of all mice dying at the 6000 ppm level. Data from these animals were not included in the William's test.

vesicle, brain, and adrenal weights. However, all differences were not statistically significant and in most cases decreases reflect depressed growth and not organ-specific changes. Liver weights of mice in the 3000 ppm groups, with the exception of male mice receiving sulfone, exceeded control values.

PATHOLOGY

The histologic changes which were considered to be compound related are summarized in Tables 58-64. Tables in Appendix B contain an alphabetical listing of all compound-related and spontaneous changes observed.

The changes observed during necropsy of the rats and mice are presented in tables in Appendix B. Changes which were frequently observed in rats and mice during necropsy included generalized reductions in body fat and variations in the overall color of the liver. The changes in overall hepatic coloration could not be correlated with microscopic lesions and were therefore interpreted to have resulted from variations in the amounts of blood removed from each animal during prenecropsy exsanguination. Focal (1 to 3 mm in diameter) discolorations of the liver surface were occasionally observed in rats and mice. Microscopic examination of the affected livers frequently revealed small areas of hepatocellular necrosis.

Compound-related lesions occurred in the livers of rats and in the livers and lungs of mice. The hepatic lesions were similar in both rats and mice.

The earliest microscopically detectable hepatic change was megalocytosis of centrilobular hepatocytes (Figure 13). A frequent finding in rats and mice with megalocytic hepatocytes was syncytium formation. Affected hepatocytes contained 2 to 16 nuclei which were peripherally arranged adjacent to the plasma membrane (Figure 14). The most severe hepatic change observed was multifocal coagulative necrosis. Areas of necrosis were variable in size and occasionally involved multiple adjacent hepatic lobules (Figure 15). The absence of megalocytosis in

TABLE 58. SUMMARY OF COMPOUND-RELATED HEPATIC LESIONS, 28-DAY RAT STUDY

Organ and Diagnosis	Compound	Control		Sulfone 3000 ppm		Sulfide 3000 ppm		Control		Sulfoxide 3000 ppm	
	Dose Group										
	Sex	M	F	M	F	M	F	M	F	M	F
	Number in Group	10	10	6	6	6	6	6	6	6	6
Liver, necrosis				1		2		1			
Liver, megalocytosis				3	3	3				6	6
Liver, vacuolar cytoplasmic change						1					

TABLE 59. SUMMARY OF COMPOUND-RELATED HEPATIC LESIONS, 91-DAY RAT STUDY

Organ and Diagnosis	Compound	Control		Sulfone 3000 ppm		Sulfone 1500 ppm		Sulfone 750 ppm		Sulfide 3000 ppm		Sulfide 1500 ppm		Sulfide 750 ppm		Control		Sulfoxide 3000 ppm		Sulfoxide 1500 ppm		Sulfoxide 750 ppm	
	Dose Group																						
	Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
	Number in Group	14	14	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Liver, necrosis							1				1			2				3		1			
Liver, megalocytosis				9	10	10	10	9	10	10	9	10	10	10	10			7	9	10	10	10	10
Liver, vacuolar cytoplasmic change						1	5		10				5	1	5				2		8		7
Liver, sinusoidal ectasia									1														

TABLE 60. SUMMARY OF COMPOUND-RELATED HEPATIC LESIONS, RAT 91-DAY RECOVERY GROUP

Organ and Diagnosis	Compound	Sulfone		Sulfide		Sulfoxide	
	Dose Group	3000 ppm		3000 ppm		3000 ppm	
	Sex	M	F	M	F	M	F
	Number in Group	3	3	3	3	5	3
Liver, necrosis				1			
Liver, megalocytosis		2		3		3	
Liver, vacuolar cytoplasmic change			1			2	1

TABLE 61. SUMMARY OF COMPOUND-RELATED HEPATIC LESIONS, EARLY DEATHS IN MOUSE STUDY GROUPS

Organ and Diagnosis	Study	28 Day		28 Day Recovery		63 Day		63 Day Recovery		28 Day		63 Day		91 Day	
	Compound	Sulfide		Sulfide		Sulfide		Sulfide		Sulfoxide		Sulfoxide		Sulfoxide	
	Dose Group	6000 ppm		6000 ppm		6000 ppm		6000 ppm		5000 ppm		5000 ppm		5000 ppm	
	Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F
	Number in Group	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Liver, megalocytosis			1	1	1	1		1							
Liver, vacuolar cytoplasmic change				1	1										
Liver, basophilia of cytoplasm												1		1	

TABLE 62. SUMMARY OF COMPOUND-RELATED HEPATIC LESIONS, 28-DAY MOUSE STUDY

Organ and Diagnosis	Compound	Control		Sulfone		Sulfide		Control		Sulfoxide	
	Dose Group			6000 ppm		3000 ppm				3000 ppm	
	Sex	M	F	M	F	M	F	M	F	M	F
	Number in Group	9	10	5	6	7	5	6	6	6	6
Liver, necrosis		1	1	1						2	2
Liver, megalocytosis				5	3	4	3			5	5
Liver, vacuolar cytoplasmic change				1	2	1	1				

TABLE 63. SUMMARY OF COMPOUND-RELATED HEPATIC AND PULMONARY LESIONS, 91-DAY FEEDING STUDY, MOUSE

Organ and Diagnosis	Compound	Control		Sulfone		Sulfone		Sulfone		Sulfide		Sulfide		Sulfide		Control		Sulfoxide		Sulfoxide		Sulfoxide	
	Dose Group			3000 ppm		1500 ppm		750 ppm		3000 ppm		1500 ppm		750 ppm				3000 ppm		1500 ppm		750 ppm	
	Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
	Number in Group	13	13	10	10	10	9	10	10	10	10	10	9	9	11	11	9	10	10	10	10	10	10
Liver, necrosis				2						3	1							7	3				
Liver, megalocytosis				4	4	4		1	1	6	4	3		1				8	7	3		1	1
Liver, vacuolar cytoplasmic change				3	2						1					3							
Lung, bronchial and bronchiolar epithelial degeneration				10	10						3							2					

TABLE 64. SUMMARY OF COMPOUND-RELATED HEPATIC LESIONS, MOUSE 91-DAY RECOVERY GROUP

Organ and Diagnosis	Compound	Sulfone		Sulfide		Sulfoxide	
	Dose Group	3000 ppm		3000 ppm		3000 ppm	
	Sex	M	F	M	F	M	F
	Number in Group	3	3	2	1	4	4
Liver, necrosis							
Liver, megalocytosis		3	3	2		4	4
Liver, vacuolar cytoplasmic change				1			

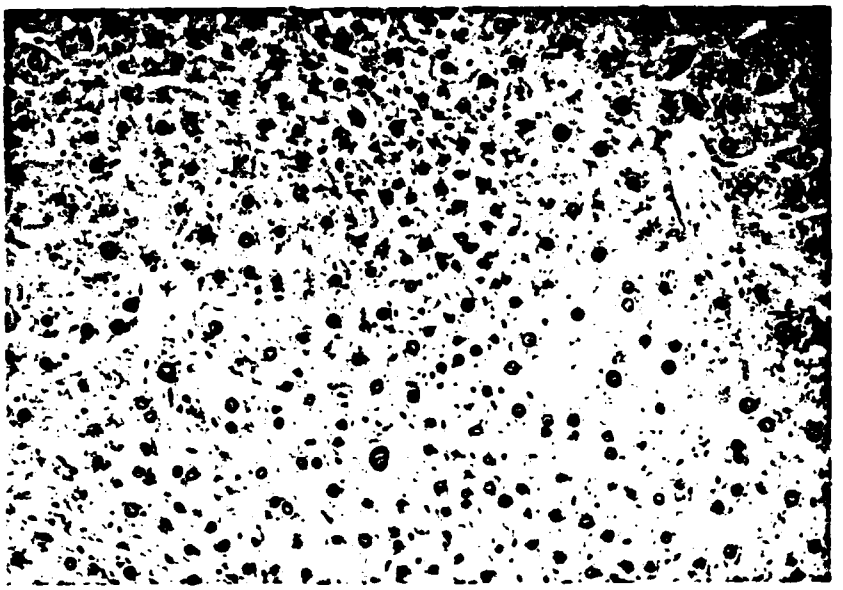


FIGURE 13. ANIMAL NUMBER 1409, MOUSE LIVER, SULFOXIDE, 3000 ppm, 91 DAYS

The earliest microscopically detectable hepatic change was megalocytosis of centrilobular hepatocytes. Small periportal hepatocytes are bounded by centrilobular areas on the right and left.

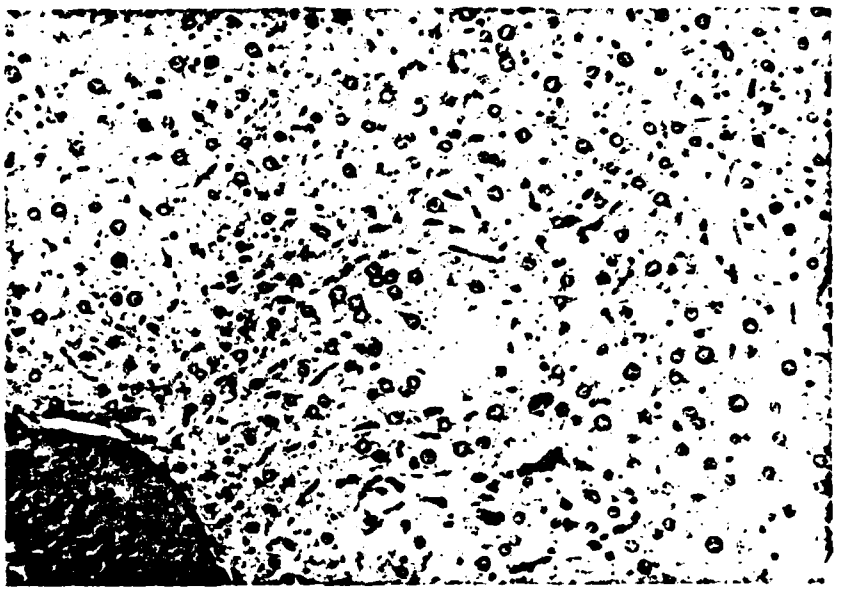


FIGURE 14. ANIMAL NUMBER 1003, MOUSE LIVER, SULFONE, 3000 ppm, 91 DAYS

A frequent finding in livers of both rats and mice with megalocytic hepatocytes was syncytium formation. Affected hepatocytes contained 2 to 16 nuclei which were usually located adjacent to the cell membrane.

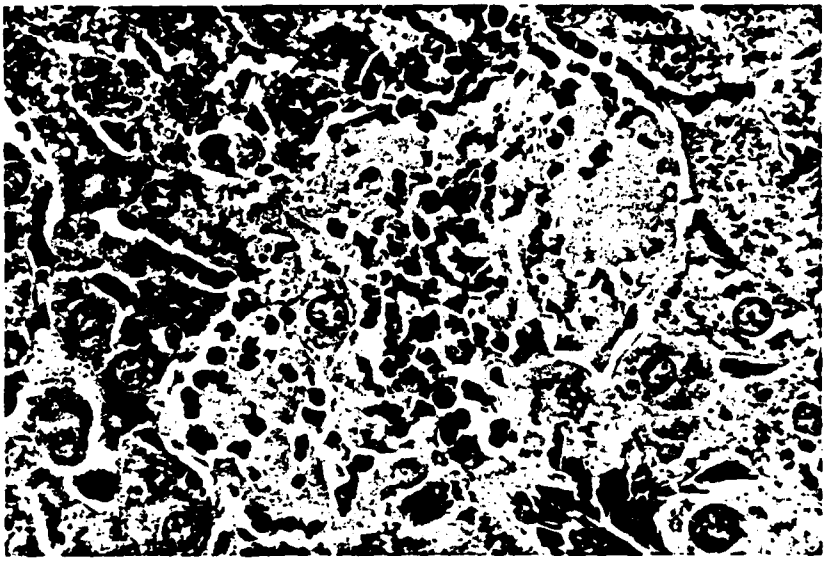


FIGURE 15. ANIMAL NUMBER 1410, MOUSE LIVER, SULFOXIDE, 3000 ppm, 91 DAYS

Early necrotizing lesion with a predominantly mononuclear cell inflammatory reaction.

association with hepatic necrosis in one control rat (number 784535) and two control mice (numbers 782732 and 782741) suggests a different underlying pathogenic mechanism for this lesion in these animals as hepatic necrosis occurred concomitantly with megalocytosis in compound-treated rats and mice. There was a dose response relationship for hepatic necrosis and megalocytosis in mice receiving all three compounds. The incidence of hepatic lesions in mice was highest in the sulfoxide-treated groups. A similar finding was also observed for hepatic necrosis in the rats receiving sulfoxide. The incidence of megalocytosis in rats treated for 91 days with each dose level of the three compounds varied from 80 to 100%.

Vacuolar cytoplasmic change was a frequently observed hepatic lesion (Figure 16). This change was observed in both control rats and mice; however, the increased incidence of this change in treated animals was considered to be a compound-related effect.

A compound-related pulmonary lesion was observed in mice. This change was confined to the respiratory epithelium of the bronchi and bronchioles and consisted of denudation and/or flattening of the lining epithelium. Occasionally the affected epithelial cells were squamoid in appearance and varied from one to two cell layers in depth. These pulmonary changes were seen in all mice fed 3000 ppm sulfone for 91 days. Mice receiving sulfide and sulfoxide at the 3000 ppm dose level were affected at a rate of 15 and 16%, respectively. Pulmonary tissue from the 1500 and 750 ppm dose groups for the three compounds was not histologically evaluated.

Bronchiolar papillary hyperplasia was observed in one mouse from the high dose sulfoxide-treated group. The occurrence of this lesion in a single animal presents a clear interpretation of its relationship to treatment.

The other histologic lesions which were observed in this study were lesions which were observed in the control groups and/or were changes which are commonly found in untreated, laboratory-reared rats and mice and were interpreted as spontaneous changes.

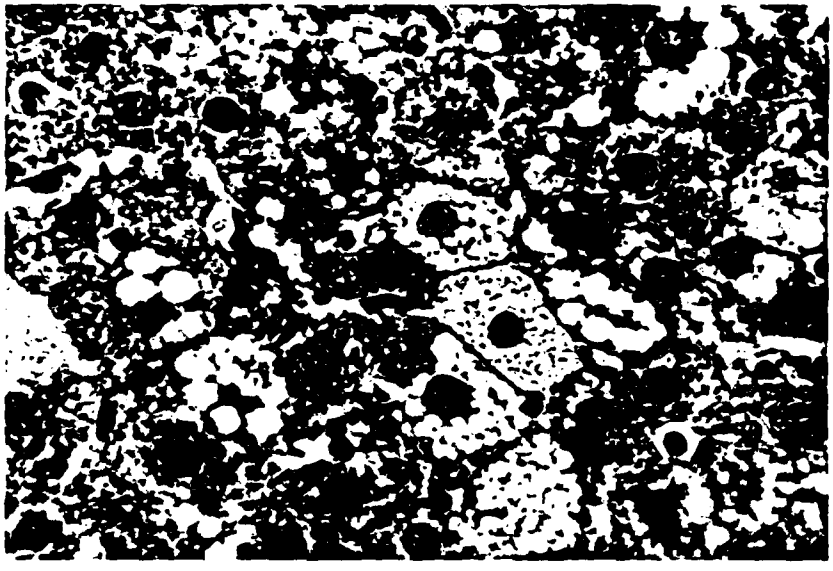


FIGURE 16. ANIMAL NUMBER 1683, RAT LIVER, SULFOXIDE, 750 ppm, 91 DAYS

Vacuolar cytoplasmic change of hepatocytes.

14-DAY ORAL GAVAGE STUDIES IN RHESUS MONKEYS

METHODS

P-chlorophenyl methyl sulfide, sulfoxide, and sulfone were administered daily to rhesus monkeys by oral gavage for 14 consecutive days. One-half of the monkeys were terminated on day 15 and the remainder were terminated following a 14-day recovery period. This study was originally designed to include three dosage levels of each compound to include (1) a high toxic dose, (2) a low toxic dose, and (3) a maximum tolerated dose. These dosage levels would have had to be established initially and then additional monkeys added at the appropriate levels. Rhesus monkeys became impossible to obtain due to export restrictions imposed by the country of origin. Only enough monkeys were available to complete the metabolism and pharmacokinetic studies and provide 18 monkeys per compound plus six controls for toxicity studies. Therefore, it was not possible to follow the original design criteria. In order to attain large enough numbers in the various groups for statistical purposes, all animals from lethal dose levels for a given compound (where more than one existed) were included as one group. Similar combinations were made for other groups where more than one level was used in attempts to establish no effect levels.

Three male and three female monkeys were treated at each designated level (which in some instances included more than one dosage level). Prior to being placed on study, each monkey had at least two complete baseline blood profiles, one ophthalmic exam, and an ECG tracing and other standard quarantine procedures as detailed in Appendix A. All monkeys were individually housed in steel cages in a room with a light/dark cycle of 12 hours each. Temperature and humidity were also controlled. Purina Monkey Chow was fed twice daily and water was provided ad libitum. Each monkey was individually identified by a permanent tattoo on the chest; each cage was also labeled with the monkey identification number, compound, dose, and start date.

Administration of test chemicals was completed by noon each day so that adequate observations could be made throughout the remainder of

the day. Chemical administration was achieved by passing a KY jelly-lubricated infant feeding tube (B-D Infant Feeding Tube 8 fr.) through the nasal cavity and into the monkey's stomach. Each compound was formulated in 100% corn oil at the proper concentrations. Six control animals received 14 daily treatments (gavage) of 100% corn oil in as much volume (by kg body weight) as was required for the highest dose level administered to any treatment animal. The highest dose level administered was 80 mg/kg of sulfide (Table 66); therefore, a volume of corn oil equaling that dose was given orally by kg body weight to all control animals. Disposable sterile syringes were used for compound transport, and the feeding tubes were adequately flushed with 1 cc pure corn oil after the compound was administered.

Doses were adjusted according to each animal's individual weight at four different times during the 14-day treatment period (days 1, 4, 7, and 11). Blood samples were collected prior to treatment and on days 7 and 15 on all test animals and additionally on days 21 and 29 on the 2-week recovery animals.

On day 15, 24 hours after the final treatment, three animals per dose level were randomly selected for necropsy. The remaining three animals were observed throughout a 2-week recovery period and terminated on day 29. Prenecropsy physical examinations were performed on each monkey by a staff veterinarian. Final ophthalmic examinations were performed and ECG recordings were obtained. Complete necropsy examinations were performed by technicians trained in large animal necropsy and supervised by a pathologist. Table 65 explains the schedule followed in the 14-day oral gavage study of monkeys.

Tissues routinely examined microscopically and hematology and clinical chemistry parameters routinely evaluated are shown in Appendix A.

Pretreatment and posttreatment ophthalmic examinations were performed on monkeys given sulfone, sulfide, and sulfoxide and on control monkeys. The monkeys were immobilized on restraint boards. Two or three drops of Mydriacil (Alcon Laboratories) were instilled into the conjunctival sac of both eyes of each monkey. One-half hour after instillation of the drops, the monkey's eyes were examined using a Welch Allyn Direct Ophthalmoscope for fundoscopic examination and an American Optical Slit-Lamp

TABLE 65. SCHEDULE FOR 14-DAY ORAL GAVAGE MONKEY STUDY(a)

Day	Activity	
-10 to -15	Baseline blood profile Weight and temperature	ALL ANIMALS ↓
-1 to -5	Baseline blood profile Weight and temperature ECG Opthalmic exam	
1	Initial treatment Weight and temperature	
(2 - 14	Daily treatment)	
4	Treatment Weight and temperature Dose adjustment	
7	Treatment Blood profile Weight and temperature Dose adjustment	
11	Treatment Weight and temperature Dose adjustment	
14	Final treatment Opthalmic exam ECG Prenecropsy physical	
15	Necropsy (1/pair) Blood profile Weight and temperature	RECOVERY ANIMALS ↓
21	Weight and temperature Blood profile	
28	Opthalmic exam ECG Prenecropsy physical	
29	Necropsy Blood profile Weight and temperature	

(a) Treatment - days 1-14, Recovery - days 15-29.

Biomicroscope for examination of the iris, lens, cornea, and conjunctivae and the anterior chamber. All ophthalmic exams were performed by a veterinarian experienced in ophthalmology (H. Hugh Harroff, Jr., D.V.M., or Paul C. Stromberg, D.V.M.).

Dosage information and initial treatment and necropsy dates for all animals in this study are shown in Table 66 of the Results section.

RESULTS

Dosage levels administered, dates of initial treatment and necropsy, condition of the animal at necropsy, and the individual identification numbers for all monkeys treated orally with sulfoxide, sulfone, and sulfide are shown in Table 66. The monkeys designated as controls are included in that table.

All major clinical abnormalities occurring in monkeys from all dose levels for all compounds as well as those observed in control animals are listed in Table 67.

Mean values for hematologic parameters are shown in Tables 68, 69, and 70 for sulfone, sulfide, and sulfoxide, respectively. Mean values for clinical chemistry parameters are shown in Tables 71, 73, and 75 for sulfone, sulfide, and sulfoxide, respectively, while Tables 72, 74, and 76 show those parameters in which there was a statistically significant difference for combined male and female groups during treatment as compared to baseline values. The statistically significant changes in the sulfone group included increased BUN values and decreased glucose values for the highest dosage groups on day 7. Only two animals lived to day 14 from these dosage groups; therefore, statistical evaluation was not possible after the day 7 sampling interval. Significant changes in the sulfide group include increased BUN levels from the highest (lethal) dosage groups on days 7 and 14, increased total bilirubin on day 7 (not biologically significant), decreased glucose values in the highest dosage group on day 14, and decreased alkaline phosphatase levels in the intermediate and highest dosage groups on day 14. The BUN levels were significantly elevated in monkeys treated with sulfoxide

TABLE 66. MORTALITY DATA FOR 14-DAY ORAL GAVAGE IN MONKEYS TREATED WITH SULFOXIDE, SULFONE, AND SULFIDE AND CONTROLS

Compound	Dose, mg/kg	Initial Treatment	Necropsy Date	Necropsy Day	Condition at Necropsy	Animal ID
Sulfoxide	20.0	7-11	8-8	29	Normal	M761AM
	20.0	7-11	7-25	15	Depressed	M788AF
	20.0	8-8	9-5	29	Normal	M758BM
	20.0	8-8	8-22	15	Severely depressed	M790AF
	20.0	8-8	9-5	29	Depressed	M743BF
	20.0	8-8	8-22	15	Depressed	M767AM
	10.0	8-8	9-5	29	Normal	M781AF
	10.0	8-8	8-22	15	Normal	M763AM
	10.0	8-8	9-5	29	Normal	M759BM
	10.0	8-8	8-22	15	Normal	M779AF
	10.0	6-1	6-29	29	Normal	M782AF
	10.0	6-1	6-15	15	Normal	M771AM
	5.0	7-11	8-8	29	Normal	M787AF
	5.0	7-11	7-25	15	Normal	M765AM
	5.0	8-15	9-12	29	Normal	M784AF
	5.0	8-15	8-29	15	Normal	M772AM
	5.0	8-15	8-29	15	Normal	M703BM
	5.0	8-15	9-12	29	Normal	M789AM
	5.0	11-7	11-21	15	Normal	M885F
Sulfone	30.0	3-29	4-7	10	Moribund	M713BF
	30.0	3.29	4-7	10	Moribund	M704BM
	20.0	7-14	8-11	29	Normal	M708BM
	20.0	4-27	5-12	15	Moribund	M709BF
	20.0	4-27	5-5	9	Dead	M710BF
	15.0	6-13	7-11	29	Normal	M705BM
	15.0	6-13	6-27	15	Normal	M786AF
	10.0	6-13	7-11	29	Normal	M717BF
	10.0	6-13	6-27	15	Normal	M795AM
	10.0	6-5	7-5	29	Normal	M798AM
	10.0	6-5	6-19	15	Normal	M720BF
	10.0	4-11	5-9	29	Normal	M792AF
	10.0	4-11	4-25	15	Normal	M702BM
	5.0	6-1	6-29	29	Normal	M775AM
	5.0	6-1	6-15	15	Normal	M721BF
	2.5	5-3	5-31	29	Normal	M701BM
	2.5	5-3	5-17	15	Normal	M716BF
	2.5	6-20	7-18	29	Normal	M776AM
	2.5	6-20	7-15	15	Normal	M778AF

TABLE 66. (Continued)

Compound	Dose, mg/kg	Treatment	Necropsy Date	Necropsy Day	Condition at Necropsy	Animal ID
Sulfide	80.0	3-29	4-3	6	Dead	M794AM
	80.0	3-29	4-6	9	Moribund	M722BF
	40.0	4-27	5-5	9	Dead	M711BF
	40.0	4-27	5-5	9	Moribund	M707BM
	30.0	6-13	6-25	14	Moribund	M712BF
	30.0	6-13	6-26	14	Moribund	M796AM
	20.0	6-13	6-28	16	Dead	M799AM
	20.0	6-13	6-25	13	Moribund	M719BF
	20.0	6-5	6-16	12	Dead	M773AM
	20.0	6-5	6-18	14	Moribund	M725BF
	20.0	4-11	5-9	29	Normal	M797AM
	20.0	4-11	4-25	15	Depressed	M724BF
	10.0	6-1	6-29	29	Normal	M793AM
	10.0	6-1	6-15	15	Normal	M718BF
	10.0	7-18	8-15	29	Normal	A741BF
	10.0	7-18	8-1	15	Normal	M762AM
	10.0	7-18	8-15	29	Normal	M764AM
	10.0	7-18	8-1	15	Normal	M777AF
	5.0	5-3	5-31	29	Normal	M774AM
	5.0	5-3	5-17	15	Normal	M723BF
	5.0	6-20	7-18	29	Normal	M768AM
	5.0	6-20	7-5	15	Normal	M780AF
	5.0	7-18	8-15	29	Normal	M785AF
	5.0	7-18	8-1	15	Normal	M746BM
	0.0	7-13	8-8	29	Normal	M756BM
	0.0	7-13	7-27	15	Normal	M736BF
	0.0	7-11	8-8	29	Normal	M742BF
	0.0	7-11	7-25	15	Normal	M750BM
	0.0	4-25	5-23	29	Normal	M791AF
	0.0	4-25	5-9	15	Normal	M706BM

TABLE 67. ABNORMAL CLINICAL OBSERVATIONS FOR RHESUS MONKEYS, 14-DAY ORAL GAVAGE STUDY

Compound	Dose, mg/kg	Anorexia	Adipsia	Emesis	Hypothermia	Depression Weakness	Diarrhea	Moribund or Dead at Necropsy
Sulfide						✓		✓
	80.0	✓	✓	✓		✓	✓	✓
	40.0	✓	✓		✓	✓	✓	✓
	30.0	✓			✓	✓	✓	✓
	20.0	✓			✓	✓		
	10.0	✓		✓		✓		
	5.0	✓						
Sulfone	30.0	✓	✓	✓		✓		
	20.0	✓	✓	✓	✓	✓	✓	✓
	15.0	✓		✓	✓		✓	
	10.0	✓		✓			✓	
	5.0	✓					✓	
	2.5	✓					✓	
Sulfoxide	20.0	✓		✓	✓	✓		
	10.0	✓		✓	✓		✓	
	5.0						✓	
Control	0.0			✓			✓	

TABLE 68. HEMATOLOGY VALUES FOR MALE AND FEMALE (COMBINED) MONKEYS-SULFONE (GROUP MEANS)

Dosage group, mg/kg	Pretreatment 0.0	2.5	5.0, 10.0 15.0	20.0 30.0
HCT (%)	41.00	44.00	41.00	39.00
HGB (g%)	12.60	13.00	13.10	12.70
RBC (10^6/cumm)	5.52	5.38	5.56	5.43
WBC (10^3/cumm)	7.90	5.00	6.20	5.90
Retic (%/RBC)	1.50	1.70	1.50	1.30
Platelet (10^3/cumm)	168.30	230.40	251.00	260.10
			Day 7	
HCT	40.00	38.00	39.00	38.00
HGB	12.40	11.60	12.20	11.90
RBC	5.21	4.92	5.15	5.12
WBC	9.00	6.00	6.60	11.10
Retic	1.20	1.90	1.70	1.40
Platelet	241.30	224.50	223.70	288.40
			Day 14	
HCT	38.00	38.00	38.00	36.00
HGB	11.70	11.80	11.90	11.50
RBC	4.89	4.92	5.06	5.05
WBC	8.40	5.10	8.50	7.10
Retic	2.00	2.10	1.10	1.20
Platelet	222.30	227.00	295.00	298.00
			Day 21	
HCT	39.00	38.00	38.00	40.00
HGB	12.10	11.90	11.90	13.60
RBC	5.20	5.02	5.01	5.64
WBC	7.70	5.50	5.40	4.50
Retic	2.20	1.60	1.90	1.20
Platelet	230.30	237.50	263.80	330.00
			Day 29	
HCT	40.00	39.00	39.00	40.00
HGB	12.00	12.40	12.20	12.90
RBC	5.14	5.14	5.14	5.72
WBC	8.80	6.30	6.50	9.00
Retic	1.40	2.00	2.30	2.20
Platelet	183.30	197.50	238.60	163.00

TABLE 69. HEMATOLOGY VALUES FOR MALE AND FEMALE (COMBINED) MONKEYS-SULFIDE (GROUP MEANS)

Dosage group, mg/kg	0.0	5.0	10.0	20.0, 30.0 40.0, 80.0
		Pretest		
HCT (%)	41.00	40.00	40.00	40.00
HGB (g%)	12.60	12.60	12.50	13.20
RBC (10^6/cumm)	5.52	5.31	5.42	5.58
WBC (10^3/cumm)	7.90	4.50	7.80	6.90
Retic (% RBC)	1.50	1.90	1.70	1.50
Platelet (10^3/cumm)	168.30	263.30	242.90	233.50
		Day 7		
HCT	40.00	37.00	38.00	37.00
HGB	12.40	11.50	12.20	11.80
RBC	5.21	4.85	5.19	5.07
WBC	9.00	4.80	5.90	8.40
Retic	1.20	1.80	1.50	1.00
Platelet	241.30	184.30	216.20	252.80
		Day 14		
HCT	38.00	39.00	39.00	39.00
HGB	11.70	12.20	11.90	12.30
RBC	4.89	5.04	5.23	5.22
WBC	8.40	4.40	6.80	9.30
Retic	2.00	1.50	1.50	1.10
Platelet	222.30	324.00	303.80	303.80
		Day 21		
HCT	39.00	39.00	38.00	37.00
HGB	12.10	12.10	12.00	11.70
RBC	5.20	5.05	5.21	4.83
WBC	7.70	3.90	8.70	8.70
Retic	2.20	1.60	0.70	0.90
Platelet	230.30	326.70	350.00	265.00
		Day 29		
HCT	40.00	39.00	38.00	37.00
HGB	12.00	12.30	12.00	12.20
RBC	5.14	5.15	5.11	5.08
WBC	8.80	4.10	9.30	7.80
Retic	1.40	2.20	5.30	0.70
Platelet	183.30	218.00	227.00	200.00

TABLE 70. HEMATOLOGY VALUES FOR MALE AND FEMALE (COMBINED) MONKEYS-SULFOXIDE (GROUP MEANS)

Dosage group, mg/kg	0.0	5.0	10.0	20.0
		Pretest		
HCT (%)	41.00	41.00	43.00	41.00
HGB (g%)	12.60	12.70	13.40	12.70
RBC (10^6/cumm)	5.52	5.58	5.69	5.35
WBC ($10/^3$cumm)	7.90	8.90	4.90	7.20
Retic (% RBC)	1.50	1.90	1.60	1.90
Platelet (10^3/cumm)	168.30	251.80	242.80	256.80
		Day 7		
HCT	40.00	40.00	40.00	38.00
HGB	12.40	12.20	12.30	12.00
RBC	5.21	5.29	5.23	5.04
WBC	9.00	8.50	5.00	7.70
Retic	1.20	1.90	1.70	1.50
Platelet	241.30	192.30	250.80	259.80
		Day 14		
HCT	38.00	38.00	37.00	36.00
HGB	11.70	12.10	11.70	11.50
RBC	4.89	5.21	4.98	4.86
WBC	8.40	9.70	6.90	11.20
Retic	2.00	2.10	1.20(a)	1.00(a)
Platelet	222.30	202.40	371.50	349.70
		Day 21		
HCT	39.00	39.00	36.00	37.00
HGB	12.10	12.40	11.50	11.80
RBC	5.20	5.34	4.79	4.92
WBC	7.70	10.10	4.80	4.70
Retic	2.20	2.00	1.50	1.60
Platelet	230.30	317.70	320.30	320.30
		Day 29		
HCT	40.00	41.00	37.00	38.00
HGB	12.00	12.70	11.40	11.70
RBC	5.14	5.47	4.84	5.01
WBC	8.80	25.50	5.80	4.00
Retic	1.40	2.40	2.60	1.80
Platelet	183.30	231.70	337.70	264.30

(a) Statistically significantly different from control by use of the William's test or its nonparametric equivalent, $p < 0.05$. (Statistical analysis done only on days 7 and 14.)

TABLE 71. CLINICAL CHEMISTRY VALUES FOR MALE AND FEMALE (COMBINED) MONKEYS-SULFONE (GROUP MEANS)

Dosage Groups (mg/kg)	BUN (mg/%)	Glucose (mg/%)	BSP %Ret/15 min	Alk Phos (I.U.)	SGOT (I.U.)	SGPT (I.U.)	Protime (Sec.)	Creat. (mg/%)	T.Bili (mg/%)	Na (meq/L)	K (meq/L)	Ca (meq/L)	LDH (IU)	Total Protein (g%)	Inorg. Phos. (mg%)
						Pretreatment									
0.0	19	104	12.9	380	20	19	11.2	1.0	0.3	148	3.6	5.1	167	7.2	4.4
2.5	20	114	17.9	359	23	23	12.1	1.2	0.3	150	3.8	5.5	176	7.6	5.2
5.0,10.0,15.0	17	99	23.5	346	25	15	11.0	1.0	0.2	149	4.1	5.2	178	7.3	5.4
20.0,30.0	19	115	30.3	223	28	29	11.5	1.2	0.2	148	4.1	5.2	206	7.4	5.5
						Day 7									
0.0	17	101	9.0	431	21	14	11.1	0.8	0.2	148	3.7	4.8	236	7.0	3.8
2.5	17	113	9.0	269	30	32	11.5	1.0	0.3	148	4.0	5.2	237	7.2	3.4
5.0,10.0,15.0	17	90	20.5	284	22	22	10.8	1.0	0.2	146	3.6(a)	4.9	227	7.1	4.7
20.0,30.0	38(a)	119	14.6	206	18	30	11.4	0.9	0.2	154	3.7(a)	4.8	209	7.3	3.9
						Day 14									
0.0	18	99	10.2	402	22	18	11.4	1.0	0.2	148	3.7	5.1	187	7.1	3.7
2.5	16	95	12.0	299	26	18	11.4	1.4	0.2	147	3.9	5.1	259	7.3	3.8
5.0,10.0,15.0	23	87	13.0	257	26	22	11.2	1.0	0.2	145	4.2	5.0	199	7.2	4.9
20.0,30.0	64	33(a)	25.0	187	68	18	12.4	0.9	0.3	149	4.1	4.4	180	6.8	9.0(a)
						Day 21									
0.0	19	89	17.0	407	21	13	11.2	0.9	0.3	148	3.5	6.5	224	7.4	4.4
2.5	19	111	18.0	300	23	20	10.7	0.7	0.2	146	4.1	4.6	244	7.3	5.0
5.0,10.0,15.0	16	121	17.2	251	24	21	10.9	1.0	0.2	148	4.1	5.3	181	7.6	4.6
20.0,30.0	16	77	5.0	413	25	10	10.4	1.0	0.2	148	4.1	5.2	449	7.7	4.8
						Day 29									
0.0	19	110	15.0	373	26	12	11.2	0.8	0.2	150	3.8	5.4	212	7.4	3.4
2.5	19	110	13.0	337	27	19	11.1	0.9	0.3	148	4.1	4.7	246	7.3	4.7
5.0,10.0,15.0	16	99	18.4	282	25	19	10.9	0.9	0.2	148	4.0	4.7	225	7.4	4.5
20.0,30.0	23	74	10.0	335	18	5	10.9	0.8	0.3	148	4.5	6.6	151	7.6	4.4

(a) Statistically significantly different from pretreatment by use of the William's test or its nonparametric equivalent, $p < 0.05$. (Statistical analysis done only on days 7 and 14.)

TABLE 72. STATISTICALLY SIGNIFICANTLY DIFFERENT MEAN CLINICAL CHEMISTRY VALUES FOR MALE AND FEMALE MONKEYS (COMBINED)- SULFONE

	Dosage Groups, mg/kg			
Parameter	0.0	2.5	5.0,10.0 15.0	20.0 30.0
	Pretreatment			
BUN, mg %	19	20	17	19
Glucose, g %	104	114	99	115
	Day 7			
BUN, mg %	17	17	17	38(a)
Glucose, g %	99	95	87	33(a)

(a) Statistically significantly different from pretreatment by use of the William's test or its nonparametric equivalent, $p < 0.05$. (Statistical analysis done only for days 7 and 14.)

TABLE 73. CLINICAL CHEMISTRY VALUES FOR MALE AND FEMALE (COMBINED) MONKEYS-SULFIDE (GROUP MEANS)

Dosage Groups (mg/kg)	BUN (mg/%)	Glucose (mg/%)	BSP %Ret/15 min	Alk Phos (I.U.)	SGOT (I.U.)	SGPT (I.U.)	Protime (Sec.)	Creat. (mg/%)	T.Bili (mg/%)	Na (meq/L)	K (meq/L)	Ca (meq/L)	LDH (IU)	Total Protein (g%)	Inorg. Phos. (mg%)
Pretreatment															
0.0	19	104	12.9	381	20	19	11.2	1.0	0.3	148	3.6	5.1	167	7.2	4.4
5.0	18	109	17.8	351	22	20	11.7	1.0	0.3	150	4.0	5.3	204	7.5	5.7
10.0	21	101	15.8	369	22	13	10.9	0.9	0.3	147	3.8	5.4	169	7.1	3.9
20.0, 30.0, 40.0, 80.0	17	115	20.1	375	25	27	11.4	1.1	0.2	148	4.1	5.2	198	7.5	5.4
Day 7															
0.0	17	101	9.0	431	21	14	11.1	0.8	0.2	148	3.7	4.8	236	7.0	3.8
5.0	18	113	14.8	302	26	19	11.9	1.1	0.2	147	3.9	5.1	296	7.0	4.0
10.0	16	99	13.2	389	25	9	11.3	1.1	0.2	144	3.6	5.2	180	7.1	4.2
20.0, 30.0, 40.0, 80.0	30(a)	105	17.9	259	21	34	11.7	0.9	0.3(a)	148	3.7	4.8	217	7.2	4.8
Day 14															
0.0	18	99	10.2	402	22	18	11.4	1.0	0.2	148	3.7	5.1	187	7.1	3.7
5.0	16	133	12.3	323	35	22	11.5	1.3	0.2	146	4.1	5.6	303	7.1	4.2
10.0	32	93	15.5	288(a)	30	12	11.0	1.0	0.2	145	3.9	5.7	216	7.3	4.6
20.0, 30.0, 40.0, 80.0	69(a)	51(a)	20.0	203(a)	59	28	11.8	1.4	0.3	151	4.7	4.7	402	7.1	8.1
Day 21															
0.0	19	89	17	409	21	13	11.2	0.9	0.3	148	3.5	6.5	224	7.4	4.4
5.0	20	118	14	294	18	10	11.7	1.0	0.2	147	4.0	5.1	152	7.4	5.0
10.0	22	103	12	282	40	17	10.7	1.0	0.2	146	4.1	5.1	184	7.2	4.6
20.0, 30.0, 40.0, 80.0	20	116	10	194	54	36	11.9	0.7	0.2	142	4.5	4.6	383	7.7	4.9
Day 29															
0.0	19	110	15	373	26	12	11.2	0.8	0.2	150	3.8	5.4	212	7.4	3.4
5.0	18	109	17	302	25	12	11.7	1.0	0.2	148	3.9	4.9	275	7.2	4.8
10.0	16	86	5	342	21	18	10.1	0.8	0.3	145	4.1	4.8	473	7.1	3.3
20.0, 30.0, 40.0, 80.0	21	91	13	162	34	23	11.8	0.8	0.1	146	4.3	5.3	497	7.5	1.8

(a) Statistically significantly different from pretreatment by use of the William's test or its nonparametric equivalent, $p < 0.05$. (Statistical analysis done only on days 7 and 14.)

TABLE 74. STATISTICALLY SIGNIFICANTLY DIFFERENT MEAN CLINICAL CHEMISTRY VALUES FOR MALE AND FEMALE (COMBINED) MONKEYS-SULFIDE

Parameter	Dosage Groups, mg/kg 0.0	5.0	10.0	20.0,30.0 40.0,80.0
	Pretreatment			
BUN, mg %	19	18	21	17
Glucose, g %	104	109	101	115
Alk. Phos., I.U.	381	351	369	375
Total Bili, mg %	0.2	0.2	0.2	0.2
	Day 7			
BUN, mg %	17	18	16	30(a)
Total Bili, mg %	0.2	0.2	0.2	0.3(a)
	Day 14			
BUN, mg %	18	16	32	69(a)
Glucose, g %	99	133	93	51(a)
Alk. Phos., I.U.	402	323	288(a)	203(a)

(a) Statistically significantly different from pretreatment by use of the William's test or its nonparametric equivalent, $p < 0.05$. (Statistical analysis done only for days 7 and 14.)

TABLE 75. CLINICAL CHEMISTRY VALUES FOR MALE AND FEMALE (COMBINED) MONKEYS-SULFOXIDE (GROUP MEANS)

Dosage Groups (mg/kg)	BUN (mg/%)	Glucose (mg/%)	BSP %Ret/15 min	Alk Phos (I.U.)	SGOT (I.U.)	SGPT (I.U.)	Protime (Sec.)	Creat. (mg/%)	T.Bili (mg/%)	Na (meq/L)	K (meq/L)	Ca (meq/L)	LDH (IU)	Total Protein (g%)	Inorg. Phos. (mg%)
						Pretreatment									
0.0	19	104	12.9	381	20	19	11.2	1.0	0.3	148	3.6	5.1	167	7.2	4.4
5.0	19	99	11.9	346	21	12	11.1	1.0	0.2	151	4.0	5.2	167	7.1	4.8
10.0	16	105	14.9	354	22	13	10.9	1.1	0.2	148	3.8	5.1	253	7.2	4.8
20.0	16	89	17.4	369	29	11	10.9	0.9	0.2	146	3.7	5.1	359	7.0	5.1
						Day 7									
0.0	17	101	9.0	431	21	14	11.1	0.8	0.2	148	3.7	4.8	236	7.0	3.8
5.0	14	81	8.0	340	22	19	11.0	1.2	0.2	150	3.9	4.8	191	7.0	5.2
10.0	16	93	9.5	327	22	13	10.9	1.1	0.3	147	3.7	5.5	215	7.4	4.0
20.0	25[a]	103	16.5	303	30	9	10.9	0.9	0.3	149	3.6	5.0	341	7.4	4.8
						Day 14									
0.0	18	99	10.2	402	22	18	11.3	1.0	0.2	148	3.7	5.1	187	7.1	3.7
5.0	19	90	6.0	349	21	10	11.1	1.0	0.2	149	3.8	5.6	176	7.5	4.8
10.0	31[a]	91	9.3	272[a]	31	30	11.5	0.9	0.3	148	3.8	4.9	240	7.5	5.7
20.0	35[a]	85	24.9	261[a]	33	11	10.6	0.8	0.3	144	4.3	4.7	405	7.3	5.3
						Day 21									
0.0	19	89	17.0	407	21	13	11.2	0.9	0.3	148	3.5	6.5	224	7.4	4.4
5.0	20	95	9.7	337	20	8	11.0	0.7	0.2	150	4.1	5.8	153	7.6	5.3
10.0	20	113	5.0	313	17	11	11.5	0.9	0.2	147	3.9	5.7	148	7.8	4.0
20.0	18	90	10.4	312	27	8	10.6	0.8	0.2	146	4.2	5.5	282	7.2	5.2
						Day 29									
0.0	19	110	15.0	373	26	12	11.2	0.8	0.2	150	3.8	5.4	212	7.4	3.4
5.0	21	111	14.0	324	18	12	11.3	0.8	0.3	149	3.8	5.5	202	7.4	4.1
10.0	23	86	7.3	367	21	9	10.9	0.7	0.4	149	3.9	5.5	196	7.7	3.6
20.0	19	110	9.3	338	28	6	10.5	0.8	0.2	150	4.1	5.2	347	7.7	5.1

(a) Statistically significantly different from pretreatment by use of the William's test or its nonparametric equivalent, $p < 0.05$. (Statistical analysis done only on days 7 and 14.)

TABLE 76. STATISTICALLY SIGNIFICANTLY DIFFERENT MEAN CLINICAL CHEMISTRY VALUES FOR MALE AND FEMALE (COMBINED) MONKEYS-SULFOXIDE

Parameter	Dosage Groups, mg/kg			
	0.0	5.0	10.0	20.0
	Pretreatment			
BUN, mg %	19	19	16	16
Alk. Phos., I.U.	381	346	354	369
	Day 7			
BUN, mg %	17	14	16	25(a)
	Day 14			
BUN, mg %	18	19	31(a)	35(a)
Alk. Phos., I.U.	402	349	272(a)	261(a)

(a) Statistically significantly different from pretreatment by use of the William's test or its nonparametric equivalent, $p < 0.05$. (Statistical analysis done only for days 7 and 14.)

at the highest level on day 7 and at the intermediate and highest levels on day 14. Alkaline phosphatase was significantly decreased on day 14 in monkeys given sulfoxide at the two highest levels.

The only hematologic parameter which was statistically different from baseline was the reticulocyte count which was elevated in sulfoxide-treated monkeys at the intermediate and highest levels on day 14.

In addition to the statistically significant changes, it is useful, in studies with only a few animals per group, to consider changes from baseline values which occur during or following treatment in individual animals and which are considered to be biologically significant but which are not necessarily statistically significant in group values. A discussion of those changes is considered below in the discussion of changes for each individual compound. These changes are reflected in Tables 77, 78, and 79.

Common Control

Abnormal clinical signs observed in the control animals were emesis and diarrhea. These conditions were attributed to the corn oil administered. These abnormalities are listed in Table 67.

Hematology and clinical chemistry parameters fluctuated somewhat in the control animals (Table 77). All parameters which were abnormal were back within their baseline limits by the time of necropsy except for one male who had a markedly decreased inorganic phosphorus level on days 15 and 29.

Sulfoxide

Three male and three female monkeys were treated orally with sulfoxide at each of three dosage levels: 20.0, 10.0, and 5.0 mg/kg. No spontaneous deaths occurred during the treatment or recovery periods. Four of the six monkeys at the highest dose level (20.0 mg/kg) were necropsied in depressed or severely depressed conditions (Table 77).

Anorexia, emesis, hypothermia, depression, and weakness were noted at the 20.0 mg/kg level and the same symptoms except for weakness

TABLE 77. HEMATOLOGY AND CLINICAL CHEMISTRY PARAMETERS FOR SULFOXIDE-TREATED AND CONTROL MONKEYS

Compound	Dose, mg/kg	0.0 Direction	Degree	No. of Animals Involved	5.0 Direction	Degree	No. of Animals Involved	10.0 Direction	Degree	No. of Animals Involved	20.0 Direction	Degree	No. of Animals Involved
Hematology	Hct	↓	Mod.	1 (M)	↓	Mild	1 (F)	↓	Mild-Mod.	3 (2F, M)	↓	Mod.	3 (2F, M)
	Hgb	↓	Mod.	1 (M)	↓	Mod.	2 (F)	↓	Mod.-Marked	3 (2F, M)	↓	Marked	2 (M, F)
	RBC	↓	Mild	1 (M)							↓	Mod.	4 (1F, 3M)
	WBC				↑	Marked	2 (F, M)	↑	Mod.	2 (M, F)	↓/↑	Mod.-Marked	1 (M) / 2 (M, F)
	Retic	↑	Mod.	2 (M, F)	↑	Mod.	1 (M)	↑	Mod.	1 (F)			
	Platelet				↓	Marked	1 (M)				↓	Mod.	2 (M, F)
	Diff: Lymph	↓	Mod.	1 (F)	↓	Mod.	3 (2F, M)	↓	Mod.	2 (M, F)	↓	Mod.-Marked	6 (3M, 3F)
	Diff: Neutra.	↑	Mod.		↑	Mod.		↑			↑		
Clinical Chemistry	BUN							↑	Mod.-Marked	3 (2F, M)	↑	Mod.-Marked	4 (2M, 2F)
	Glucose							↑	Mod.	4 (2M, 2F)			
	Alk PLos	↑	Mild-Mod.	1 (M)									
	SGOT	↑	Mod.	2 (M, F)	↑	Mod.	1 (F)	↑	Mod.	1 (M)	↑	Mod.	2 (M, F)
	SGPT	↑	Mod.	1 (M)	↑	Mod.	1 (M)	↑	Marked	1 (M)			
	Potassium							↓	Mod.	1 (M)			
	Calcium	↑	Mod.	1 (F)	↑	Mild-Mod.	2 (M, F)	↑	Mod.-Marked	2 (M)	↑	Mod.	2 (M)
	LDH	↑	Mod.	3 (2F, M)	↑	Mod.-Marked	2 (F)	↑	Mod.-Marked	2 (M, F)	↑	Mod.-Marked	2 (M, F)
	Inorg. Phos.	↓	Marked	1 (M)							↓/↑	Mild-Marked	1 (M) / 1 (F)

were observed at the 10.0 mg/kg dose level. Other abnormal fluctuations are listed in Table 77.

Depressions in the hemoglobin and hematocrit levels were observed in three animals; these parameters recovered sufficiently in two animals to allow for scheduled necropsy on day 29. The other animal was terminated on day 15. The red blood cell count was moderately decreased in four animals at the 20 mg/kg dose level and no recovery occurred by the date of necropsy.

The BUN value was significantly elevated in four of the six monkeys at the highest dosage level and in one-half of those at the 10.0 mg/kg dose. One male at the 20.0 mg/kg dose recovered to his BUN baseline by day 29; the BUN baselines for the other three monkeys did not return to normal. Sporadic moderate to marked elevations occurred in the SGOT, SGPT, and calcium values. One animal at the 20.0 mg/kg level had a marked increase in the LDH value on day 15. Markedly decreased LDH values occurred in one male from the 10 mg/kg dosage group on days 21 and 29. Other fluctuations are shown in Table 77.

Sulfone

A dose of 30.0 mg/kg was supralethal as both monkeys treated at that level were terminated in moribund condition on day 10 (Table 66). One female given 20 mg/kg died on day 9 while another female at that dosage level was terminated in moribund condition on day 15. The male partner survived and was terminated on day 29. (An extra female was mistakenly put in this study; the male was added at a later date.) Because lethality occurred at the 20.0 mg/kg dose level, an additional dose level of 15.0 mg/kg was used. The two monkeys at that level survived and were terminated as scheduled on days 15 and 29. Due to the scarcity of available rhesus monkeys, animals from the three highest dose levels were grouped together for statistical purposes. Six animals at the 10.0 mg/kg dose consistuted the intermediate dose group and the low dose level for statistical purposes was the combined 5.0 and 2.5 mg/kg dose group animals.

Anorexia was the most prevalent abnormal clinical sign, as seen in Table 67. Emesis and diarrhea were also experienced by several of the animals at various dose levels.

Fluctuations in hematology parameters at the high dose levels were generally not remarkable. Two monkeys from those groups had substantial increases in their WBC counts; however, such changes are common in rhesus monkeys and were probably not directly treatment related. All general fluctuations are displayed in Table 78.

Several monkeys at dosage levels of 10.0 mg/kg and higher had moderate to severe elevations in their BUN values which persisted until termination on day 15. One male at the 15.0 mg/kg level recovered to his baseline BUN value by day 29. Monkeys at 10.0, 15.0, 20.0, and 30.0 mg/kg dose levels experienced decreased glucose values which did not return to normal prior to necropsy on day 15. The SGOT values were also increased in animals from those groups and these persisted until termination on day 15. One monkey at the 20.0 mg/kg level and one monkey at the 30.0 mg/kg dose level had substantially elevated sodium values. Two monkeys at the 10.0 mg/kg dose level had substantially decreased inorganic phosphorus values which persisted until necropsy. Also, that parameter was extremely elevated in one monkey at the 20.0 mg/kg dose level. Other fluctuations in the hematology and clinical chemistry parameters for sulfone-treated monkeys are displayed in Table 78.

Sulfide

Two monkeys were treated orally with sulfide at each of three supralethal dosage levels (80.0, 40.0, and 30.0 mg/kg). At 20 mg/kg, two monkeys died and two were terminated in moribund condition. Other dose levels administered were 10.0 mg/kg and 5.0 mg/kg. The six animals at these dosage levels survived the treatment and observation periods and were necropsied as scheduled.

Frequently occurring abnormal clinical signs for monkeys that were treated with sulfide included anorexia, hypothermia, depression, weakness, and diarrhea. Other occurrences of clinical abnormalities are listed in Table 67.

TABLE 78. HEMATOLOGY AND CLINICAL CHEMISTRY PARAMETERS FOR SULFONE-TREATED MONKEYS

Compound	Dose, mg/kg	5.0 Direction	Degree	No. of Animals Involved	10.0 Direction	Degree	No. of Animals Involved	15.0 Direction	Degree	No. of Animals Involved	20.0 Direction	Degree	No. of Animals Involved	30.0 Direction	Degree	No. of Animals Involved
Hematology	Hct.		Mild	1 (F)		Mild-Mod	3 (2F, M)		Mild	1 (F)		Mild	1 (F)			
	Hgb.		Mild	1 (F)		Mod	1 (M)		Mild	1 (F)		Mod	1 (F)			
	WBC					Marked	1 (F)		Marked	1 (M)		Marked	1 (F)	↓	Mild	1 (M)
	Retic					Mild-Mod.	1 (M)									
	Platelet		Mod.	1 (F)		Marked	2 (M, F)		Mod.			Mod.	1 (F)			
	Diff Lymph						1 (F)							↓	Marked	2 (M, F)
	Neutro													↑		
	2.5*				5.0			10.0			15.0			20.0		
Clinical Chemistry	Bun								Mod.	2 (M)		Marked	2 (M, F)	↑	Marked	2 (F)
	Glucose								Mod-Marked	3 (2M, F)				↓	Marked-Severe	2 (F)
	BSP								Mod.	1 (M)		Mod.	1 (M)			
	Alk Phos								Mod.	2 (M)		Mild	1 (M)			
	SGOT		Mild	1 (M)					Mild-Mod.	1 (M)		Mild	1 (F)	↑	Marked	1 (F)
	SGPT											Mod.	1 (F)	↓	Severe	1 (F)
	Aotime															
	Creatinine								Mod.	1 (F)						
	Sodium								Mild	1 (M)				↑	Marked	1 (F)
	Patassium								Mod.	1 (M)		Mod.	2 (M, F)		Marked	1 (F)
	LDH		Mod.	1 (M)					Mild-Mod.	4 (2M, 2F)		Mod.	1 (M)	↓	Mod.	2 (M, F)
	Inorg. Phos.		Mild-Mod.	3 (2F, M)		Mild	1 (M)		Mod-Marked	2 (M, F)		Marked	1 (M)	↑	Mod-Marked	2 (M, F)
	Calcium													↓	Mod.	1 (M)
	30.0													↑		
	Bun		Marked	2 (M, F)												
	Glucose		Severe	2 (M, F)												
	BSP		Marked	1 (F)												
	SGOT		Marked	1 (F)												
	SGPT		Mod	1 (F)												
			Severe	1 (M)												
	Protime		Mod.	1 (F)												
	Sodium		Mod.	1 (M)												
	Inorg. Phos.		Marked	2 (M, F)												

* There were no abnormalities noted in the hematology parameters in the monkeys treated at 2.5 mg/kg dose.

Markedly depressed hemoglobin and hematocrit values were observed in one animal from each of the 80.0, 40.0, and 20.0 mg/kg dosage levels. Other decreases in these parameters were sporadic with complete recovery occurring before the animals were terminated. Platelet values were decreased to a varying degree in animals from the 5.0, 10.0, and 20.0 mg/kg dose groups. In all instances, there was recovery to baseline values. A trend toward absolute neutrophilia was seen in a few animals representing all dosage levels. Recovery to baseline levels was observed only at the lowest dose level, 5.0 mg/kg. Animals from all but the 5.0 mg/kg level had increased BUN values which did not recover to their baseline levels by day of necropsy. Glucose values were severely depressed in three animals from the 20.0 mg/kg dose level and in one animal from the 30.0 mg/kg dose. At the 10.0 and 20.0 mg/kg dose levels, there were several monkeys in which depression of alkaline phosphatase values occurred with little or no recovery to baseline values. Final SGOT and SGPT values were substantially elevated in animals from the 20.0 and 30.0 mg/kg dose levels. One female from the 20.0 mg/kg dose group had an extremely high creatinine value (2.8) on day 15 coincident with an increased BUN value. Several substantial elevations in LDH values occurred in animals from all dosage groups. Inorganic phosphorus levels fluctuated sporadically. All other hematology and clinical chemistry parameter variances are displayed in Table 79.

Ophthalmic Lesions

Compound-related ophthalmic lesions were not observed in the monkeys treated with the sulfone, sulfide, or sulfoxide. As can be seen in Tables 80, 81, and 82, some eye lesions were observed, but they were either considered incidental or were observed at the pretreatment examination as well as at the posttreatment examination.

Relative Organ Weights

Relative organ weights for male and female monkeys are shown in Tables 83, 84, and 85 for sulfide, sulfone, and sulfoxide, respectively.

TABLE 79. HEMATOLOGY AND CLINICAL CHEMISTRY PARAMETERS FOR SULFIDE-TREATED MONKEYS

Dose, mg/kg	5.0 Direction	Degree	No. of Animals Involved	10.0 Direction	Degree	No. of Animals Involved	20.0 Direction	Degree	No. of Animals Involved	30.0 Direction	Degree	No. of Animals Involved	40.0 Direction	Degree	No. of Animals Involved
Hematology															
Hct.	↓	Marked	1(F)	↓	Mod.	1(M)									
Hgb.	↓	Marked	2(M/F)	↓	Mod.	1(M)	↓	Mod.-Marked	4(2M/2F)				↓	Mod.-Marked	2(M/F)
WBC				↓	Mod.-Marked	2(M/F)	↓	Mod.-Marked	2(M/F)	↓	Mod.	1(M)	↓ / ↑	Marked / Marked	1(F) / 1(M)
Retic	↑ / ↓	Mod. / Mod.	2(M/F) / 2(M)	↑	Mod.-Marked	2(M)									
Platelet	↓	Marked	2(M/F)	↓	Mod.-Marked	1(M)	↓	Mod.	1(F)	↓	Marked	1(M)	↓	Mod.	1(M)
Diff. Lymph	↓	Mod.	1(M)	↓	Mod.-Marked	1(M)	↓	Mod.-Marked	5(3M/2F)	↓	Marked	2(M/F)	↓	Marked	2(M/F)
Diff. Neutra.	↓			↓			↓			↓			↓		
80.0															
Hct.	↓	Mod.	1(F)												
Hgb.	↓	Mod.	1(F)												
WBC	↓	Mod.	1(M)												
Retic															
Platelet															
Diff. Lymph	↓	Marked	1(F)												
Diff. Neutra	↓														
Clinical Chemistry															
	5.0			10.0			20.0			30.0			40.0		
BUN				↓	Mod.-Marked	3(2F/M)	↓	Mod.-Marked	5(3M/2F)	↓	Mod.-Marked	2(M/F)	↓	Marked	2(M/F)
Glucose				↓	Mod.	1(F)	↓	Marked Severe	3(2M/F)	↓	Marked	1(M)	↓	Mod.	1(F)
BSP							↓	Marked	1(M)						
Alk. Phos.	↓	Mild	2(M/F)	↓	Mod.	1(M)	↓	Marked	5(3M/2F)						
SGOT	↓	Mild-Mod.	5(3M/2F)	↓	Mod.-Marked	4(2F/2M)	↓	Mod.-Marked	3(M)	↓	Severe	1(M)			
SGPT	↓	Mod.	1(M)				↓	Mod.-Marked	4(2M/2F)	↓	Marked	1(M)	↓	Mod.	1(F)
Creatinine							↓	Marked	1(F)	↓	Marked	1(M)			
Sodium							↓	Mod.-Marked	2(M/F)	↓	Marked	1(M)			
Potassium							↑ / ↓	Mild / Mod.	1(M) / 1(F)	↓	Mod.	1(M)	↓	Mild	1(M)
Calcium				↓	Mod.	1(F)	↓ / ↓	Mild / Mod.	1(M) / 2(F)						
LDH	↓	Mod.	4(2M/2F)	↓	Mod.-Marked	3(2M/F)	↓ / ↓	Marked / Mild	3(M) / 1(M)	↓	Severe	1(M)			
Inog. Phos.	↓	Mod.-Marked	4(2M/2F)				↓	Marked	4(2M/2F)	↓	Severe	1(M)			
							↓	Marked	2(M/F)						
Protime				↓	Mild-Mod.								↓	Mod.	1(F)
BUN 80.0	↓	Marked	1(F)												
Glucose	↓	Mod.	1(F)												
SGPT	↓	Severe	1(F)												
Protime	↓	Mod.	1(F)												
LDH	↓	Mod.	1(F)												

TABLE 80. COMPOUND-RELATED EYE LESIONS IN MONKEYS TREATED WITH SULFONE

			Pre-treatment		Post-treatment	
Animal ID	Compound	Dose (Mg/kg)	Right Eye	Left Eye	Right Eye	Left Eye
M778AF	Sulfone	2.5	Normal	Normal	Normal	Normal
M776AM	Sulfone	2.5	Normal	Normal	Normal	Normal
M716BF	Sulfone	2.5	Normal	Normal	Normal	Normal
M701BM	Sulfone	2.5	Normal	Normal	Normal	Normal
M721BF	Sulfone	5.0	Normal	Normal	Normal	Normal
M775AM	Sulfone	5.0	Normal	Normal	Normal	Normal
M702BM	Sulfone	10.0	Normal	Normal	Normal	Normal
M792AF	Sulfone	10.0	Normal	Normal	Normal	Normal
M720BF	Sulfone	10.0	Normal	Normal	Normal	Normal
M798AM	Sulfone	10.0	Normal	Normal	Normal	Normal
M795AM	Sulfone	10.0	Normal	Normal	Normal	Normal
M717BF	Sulfone	10.0	Normal	Normal	Normal	Normal
M786AF	Sulfone	15.0	Normal	Normal	Normal	Normal
M705BM	Sulfone	15.0	Normal	Normal	Normal	Normal
M710BF	Sulfone	20.0	Normal	Normal	Died before final eye exam	
M709BF	Sulfone	20.0	Normal	Normal	Died before final eye exam	
M708BM	Sulfone	20.0	Normal	Normal	Normal	Normal
M704BM	Sulfone	30.0	Normal	Normal	Died before final eye exam	
M713BF	Sulfone	30.0	Normal	Normal	Died before final eye exam	

TABLE 81. COMPOUND-RELATED EYE LESIONS IN MONKEYS TREATED WITH SULFOXIDE

			Pre-treatment		Post-treatment	
Animal ID	Compound	Dose (Mg/kg)	Right Eye	Left Eye	Right Eye	Left Eye
M736BF	Sulfoxide	0.0	Normal	Normal	Normal	Normal
M756BM	Sulfoxide	0.0	Normal	Normal	Normal	Normal
M750BM	Sulfoxide	0.0	Normal	Normal	Normal	Normal
M742BF	Sulfoxide	0.0	Normal	Normal	Normal	Normal
M791AF	Sulfoxide	0.0	Normal	Normal	Normal	Normal
M706BM	Sulfoxide	0.0	Normal	Normal	Normal	Normal
M765AM	Sulfoxide	5.0	Normal	Normal	Normal	Normal
M787AF	Sulfoxide	5.0	Normal	Normal	Normal	Normal
M772AM	Sulfoxide	5.0	Normal	Normal	Normal	Normal
M784BF	Sulfoxide	5.0	Normal	Normal	Normal	Normal
M715BF	Sulfoxide	5.0	Normal	Normal	Normal	Normal
M789AM	Sulfoxide	5.0	Normal	Normal	Normal	Normal
M703BM	Sulfoxide	5.0	Normal	Normal	Normal	Normal
M771AM	Sulfoxide	10.0	Normal	Normal	Normal	Normal
M782AF	Sulfoxide	10.0	Normal	Normal	Normal	Normal
M779AF	Sulfoxide	10.0	Normal	Normal	Normal	Normal
M759BM	Sulfoxide	10.0	Normal	Normal	Normal	Normal
M763AM	Sulfoxide	10.0	Normal	Normal	Normal	Normal
M781AF	Sulfoxide	10.0	Normal	Normal	Normal	Normal
M788AF	Sulfoxide	20.0	Normal	Normal	Normal	Normal
M761AM	Sulfoxide	20.0	Normal	Normal	Normal	Normal
M790AF	Sulfoxide	20.0	Normal	Normal	Normal	Normal
M758BM	Sulfoxide	20.0	Superficial Corneal Scar	Corneal Scar/Some Neovascularization/Slight Pannus	Normal	Corneal Scar/Neovascularization/Slight Pannus
M767AM	Sulfoxide	20.0	Normal	Normal	Normal	Normal
M743BF	Sulfoxide	20.0	Normal	Superficial Corneal Scar	Normal	Normal

TABLE 82. COMPOUND-RELATED EYE LESIONS IN MONKEYS TREATED WITH SULFIDE

			Pre-treatment		Post-treatment	
Animal ID	Compound	Dose (Mg/kg)	Right Eye	Left Eye	Right Eye	Left Eye
M723BF	Sulfide	5.0	Normal	Normal	Normal	Normal
M774AM	Sulfide	5.0	Normal	Normal	Normal	Normal
M780AF	Sulfide	5.0	Normal	Normal	Normal	Normal
M768AM	Sulfide	5.0	Normal	Normal	Normal	Normal
M746BM	Sulfide	5.0	Normal	Normal	Normal	Normal
M785AF	Sulfide	5.0	Normal	Normal	Normal	Normal
M718BF	Sulfide	10.0	Normal	Normal	Normal	Normal
M793AM	Sulfide	10.0	Normal	Normal	Normal	Normal
M777AF	Sulfide	10.0	Normal	Normal	Normal	Normal
M764AM	Sulfide	10.0	Normal	Normal	Normal	Normal
M762AM	Sulfide	10.0	Normal	Normal	Normal	Normal
M741BF	Sulfide	10.0	Normal	Normal	Normal	Pannus in medial canthus
M724BF	Sulfide	20.0	Normal	Normal	Normal	Normal
M797AM	Sulfide	20.0	Normal	Normal	Normal	Normal
M725BF	Sulfide	20.0	Puncture of Cornea scar-Neovase	Normal	Puncture of Cornea-Neovase scarring	Normal
M773AM	Sulfide	20.0	Normal	Normal	Normal	Normal
M719BF	Sulfide	20.0	Normal	Normal	Died before final eye exa	
M799AM	Sulfide	20.0	Normal	Normal	Normal	Normal
M712BF	Sulfide	30.0	Normal	Normal	Died before final eye exa	
M796AM	Sulfide	30.0	Normal	Normal	Normal	Normal
M711BF	Sulfide	40.0	Normal	Normal	Died before final eye exa	
M707BM	Sulfide	40.0	Normal	Normal	Died before final eye exa	
M794AM	Sulfide	80.0	Normal	Normal	Died before final eye exa	
M722BF	Sulfide	80.0	Normal	Normal	Died before final eye exa	

TABLE 83. RELATIVE ORGAN WEIGHTS FOR MALE AND FEMALE (COMBINED) MONKEYS-SULFIDE (GROUP MEANS)

Organ	Dosage Group, mg/kg			
	0.0	5.0	10.0	20.0,30.0 40.0,80.0
Left Ventricle	1.72	1.79	1.71	1.79
Right Ventricle	0.79	0.83	0.74	0.91
IVS	1.03	0.81	1.25	1.16
Atrium	0.51	0.62	0.48	0.78(a)
Pituitary	0.02	0.01	0.02	0.02
Adrenal	0.23	0.24	0.25	0.54(a)
Thyroid	0.23	0.16	0.25	0.24
Brain	23.6	23.9	25.0	30.6(a)
Testicles	1.13	0.56	0.96	1.26
Ovaries	0.09	0.08	0.09	0.10
Liver	23.3	24.9	33.0(a)	35.9(a)
Right Kidney	2.02	1.99	2.90(a)	2.89(a)
Left Kidney	2.02	2.00	2.90(a)	2.88(a)

(a) Statistically significantly different from control by use of the William's test or its nonparametric equivalent, $p < 0.05$.

TABLE 84. RELATIVE ORGAN WEIGHTS FOR MALE AND FEMALE (COMBINED) MONKEYS-SULFONE (GROUP MEANS)

Organ	Dosage Group, mg/kg			
	0.0	2.5	5.0,10.0, 15.0	20.0, 30.0
Left Ventricle	1.72	1.79	1.81	1.77
Right Ventricle	0.79	0.69	0.77	0.78
IVS	1.03	0.92	1.06	0.94
Atrium	0.51	0.57	0.51	0.57
Pituitary	0.02	0.02	0.01	0.02
Adrenal	0.23	0.19	0.26	0.44(a)
Thyroid	0.23	0.18	0.18	0.21
Brain	23.6	24.4	24.2	28.4
Testicles	1.10	0.80	0.88	0.62
Ovaries	0.09	0.10	0.07	0.05
Liver	23.3	24.8	27.6	28.6
Right Kidney	2.02	1.83	2.21	2.44
Left Kidney	2.02	1.91	2.24	2.48

(a) Statistically significantly different from control by use of the William's test or its non-parametric equivalent, $p < 0.05$.

TABLE 85. RELATIVE ORGAN WEIGHTS FOR MALE AND FEMALE (COMBINED) MONKEYS-SULFOXIDE (GROUP MEANS)

Organ	Dosage Group, mg/kg			
	0.0	5.0	10.0	20.0
Left Ventricle	1.72	1.72	1.88	1.84
Right Ventircle	0.79	0.84	0.79	0.75
IVS	1.03	1.04	1.02	1.11
Atrium	0.51	0.66	0.67	0.67
Pituitary	0.02	0.02	0.01	0.01
Adrenal	0.23	0.28	0.21	0.33
Thyroid	0.23	0.29	0.18	0.46
Brain	23.6	22.5	25.5	26.3
Testicles	1.22	1.06	1.41	1.15
Ovaries	0.09	0.07	0.12	0.13
Liver	23.3	26.2	31.0(a)	34.1(a)
Right Kidney	2.0	2.1	2.5(a)	2.7(a)
Left Kidney	2.0	2.0	2.5(a)	2.6(a)

(a) Statistically significantly different from control by use of the William's test or its non-parametric equivalent, $p < 0.05$.

Statistically significant increases in liver and kidney weights were observed in intermediate and high dosage groups treated with sulfide and sulfoxide. Significant increases in adrenal weights occurred in the high dosage groups given sulfide and sulfone. Increases were also observed in the brain and atria (heart) of the high dosage groups treated with sulfide.

PATHOLOGY

The incidence of all lesions for all compounds is summarized in Table 86. A summary of lesions by compound, dosage group, and individual animal is shown in Appendix B. Lesions which were considered to be compound induced were observed in a number of different organs in the animals from this study and these lesions were designated in Table 86.

The most significant lesions involved the lymphoid system. These lesions consisted of nodular or diffuse proliferation of large undifferentiated lymphoreticular or histiocyte-like cells in the cortical areas of lymph nodes as well as in the white pulp of the spleen. Lesions in lymph nodes involved the germinal centers and were characterized by irregular proliferation of cells in these areas with coalescence of adjacent nodules. A rim of lymphocytes was usually present around the follicles at the periphery of the lymph nodes but was often indistinct toward the center of the nodes. Diffuse lesions were present in the lymph nodes from two animals and this involved proliferation of histiocyte-like cells in a diffuse pattern throughout the cortex which, in most lymph nodes, involved virtually the entire cortical area of the lymph nodes. The lesion in the spleen involved the white pulp and consisted of proliferations of histiocyte-like cells similar to those which were observed in the lymph nodes; these proliferative lesions were usally nodular with coalescence of adjacent nodules in more severely involved spleens. Nodular proliferations of lymphoreticular cells were also prominent in the bone marrow of an animal treated at the low dosage level of sulfoxide. Nodular lesions were present in the lymph nodes and spleen of this animal. All of the lymph nodes examined, as well as the spleen,

TABLE 86. SUMMARY OF LESIONS BY COMPOUND, DOSE GROUP, AND TERMINATION SCHEDULE FOR MONKEYS TREATED WITH p-CHLOROPHENYL METHYL SULFONE, SULFIDE, AND SULFOXIDE

Organ and Diagnosis	Compound	Sulfone						Sulfide						Sulfoxide						Control	
	Dosage Group	High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose			
	Group(a)	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
	Number in Group	4	1	5	5	2	2	11	1	3	3	3	3	3	3	3	3	4	3	3	3
Lung, lesions consistent with lung mite infestation		4	1	4	4	2	2	11	1	2	2	2	3	3	3	3	3	3	2	3	3
Lung, pneumonia, chronic active, multifocal				1																	
Lung, inhalation pneumonia							1	1													
Lung, interstitial pneumonia, multifocal with alveolar cell hyperplasia														1							
Lung, peribronchiolitis, granulomatous, focal, parasitic (lung mite)														1							
Trachea, mucosa, lymphocytic infiltrates, focal or multifocal										1				1		1					
Liver, hepatitis, chronic, eosinophilic, focal					1																
Liver, neutrophil infiltrate, focal					1																
Liver, lymphocyte infiltration, focal or multifocal					3								1	1	1						1
*Liver, hepatocyte vacuolization, diffuse				1				5		1					1		1	1	1		
Liver, hepatocyte necrosis, focal					1																
Liver, eosinophilic cytoplasmic change, multifocal								1													
*Liver, hepatocyte cytoplasm, vacuolar degeneration, multifocal or diffuse								1		1				2		1		1			
Liver, congestion, multifocal								1													
*Liver, vacuolar degeneration with necrosis, multifocal or diffuse		1						1													
*Liver, centrilobular areas, hepatocyte vacuolization											1		1		1		1				

TABLE 86. (Continued)

Organ and Diagnosis	Compound	Sulfone						Sulfide						Sulfoxide						Control	
	Dosage Group	High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose			
	Group(a)	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
	Number in Group	4	1	5	5	2	2	11	1	3	3	3	3	3	3	3	3	4	3	3	3
Liver, hepatocellular necrosis with inflammation, multifocal											1										
Liver, pericholangitis, eosinophilic, multifocal											1										
*Liver, hepatocyte necrosis, multifocal or diffuse								1		1	1										
Liver, portal areas, lymphocytic infiltrate, multifocal																			1		1
Liver, portal areas, chronic inflammation, multifocal																		1			
*Liver, megalocytosis																		1			
Liver, hepatocyte nuclei vacuolation and margination of chromatin																				1	
Liver, granuloma, focal, cystic																					1
Kidney, glomerulitis, subacute, diffuse		1																			
Kidney, interstitium, chronic inflammation				1							1										
*Kidney(s), proximal tubular epithelium, pigmentation	1		1					1	1	1				1							
Kidneys, congestion					1			1													
*Kidney(s), glomerulosclerosis								2													
*Kidneys, proximal tubular epithelium, vacuolization								1		1				1							
Right kidney, capsule, hemorrhage										1											
Right kidney, glomerulosclerosis, focal											1										
Kidneys, cortex, interstitium, lymphocytic infiltrate, focal and multifocal				3					1			1	2	1	2		1	1		2	2

TABLE 86. (Continued)

Compound	Sulfone						Sulfide						Sulfoxide						Control	
Dosage Group	High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose			
Group(a)	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
Number in Group	4	1	5	5	2	2	11	1	3	3	3	3	3	3	3	3	4	3	3	3
[illegible]'s capsule, [illegible]													1		1					
[illegible] granulomas, multifocal																	1			
[illegible], lymphoid nodules, multifocal																	1			
[illegible], bladder, mucosa, lymphoid infiltrate					1															
[illegible] muscle, degeneration	1																			
[illegible] degeneration									1				1				1			
Skeletal muscle, Sarcosporidial cyst(s)	2																			
Skeletal muscle, degeneration, multifocal	1																			
*Adrenal cortex, hyperplasia	3		2				8		1				3		1					
*Adrenal cortex, hemorrhage, focal or multifocal	3		2	1			7	1		1		1			1					
*Adrenal cortex, congestion	1						7			1			1	1	1					
*Adrenal glands, cortex, zona fasciculata, vacuolization	1						4						1		1					
*Adrenal glands, cortex, zona fasciculata, vacuolar degeneration	1						1													
Adrenal medulla, congestion			3	1	2		6		1	1	1		1	1	1				1	2
Adrenal gland, mineralization										1										
Adrenal cortices, sinusoidal ectasia with mild hemorrhage							1										1			
*Thyroid gland, follicular cell hyperplasia	1																			
Thyroid gland, lymphoid nodule, focal				1																

TABLE 86. (Continued)

Organ and Diagnosis	Compound	Sulfone						Sulfide						Sulfoxide						Control	
	Dosage Group	High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose			
	Group(a)	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
	Number in Group	4	1	5	5	2	2	11	1	3	3	3	3	3	3	3	3	4	3	3	3
*Thyroid gland, degeneration, with or without follicular cell hyperplasia and loss of thyroglobulin, focal		1						1													
Thyroid gland, cysts, multiple														1							
Parathyroid connective tissues, granuloma, foreign body, focal		1																			
*Mandibular lymph node, cortex, reticulum cell hyperplasia, multifocal		1																			
Mandibular lymph node, pigment deposition		1	1	3	2		1	2		1		2	1		3	2	2	1		1	
Mandibular lymph node, hemosiderin deposition				1	1			1		1			3	1		1	1				
Mandibular lymph node, hemorrhage with or without erythrophagocytosis		1		1	1			2		1										1	
Mandibular lymph node, lymphadenitis, eosinophilic												1									
Mesenteric lymph node, hemorrhage				1				1											1	1	
Mesenteric lymph node, parasitosis					1																
Mesenteric lymph node, fibrosis, multifocal					1																
Mesenteric lymph node, granuloma, focal					1																
Mesenteric lymph node, congestion								1													
Mesenteric lymph node, lymphadenitis, suppurative, focal								1													
Mesenteric lymph node, hemorrhage with erythrophagocytosis								1													

TABLE 86. (Continued)

Organ and Diagnosis	Compound	Sulfone						Sulfide						Sulfoxide						Control	
	Dosage Group	High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose			
	Group(a)	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
	Number in Group	4	1	5	5	2	2	11	1	3	3	3	3	3	3	3	3	4	3	3	3
Mesenteric lymph node, hemosiderin deposition													1								
Mesenteric lymph node, lymphadenitis, eosinophilic				1								1			1						
Mesenteric lymph node, medulla, histiocytosis														1							
Iliac lymph node, hemorrhage with erythrophagocytosis				1																	
Aortic lymph node, hemorrhage	1		1								1										
Bronchial lymph node, hemorrhage								1				1									
Pancreatic lymph node, hemorrhage								1													
Renal lymph node, lymphadenitis, chronic								1													
*Lymph nodes, germinal centers, lymphoid depletion	2													2							
*Lymph node(s), lymphoreticular hyperplasia			1					2										1			
*Lymph nodes, lymphoid depletion	1							2													
*Lymph nodes, hyperplasia																		1			
Spleen, neutrophilic infiltrate, multifocal or diffuse								1												1	
*Spleen, lymphoreticular hyperplasia								2										1			
*Spleen, lymphoid depletion (B cells)								2						1							
*Spleen, lymphoreticular hyperplasia	1																				
*Tonsil, lymphoid depletion								1													
*Tonsil, germinal centers, lymphocyte depletion														1							

TABLE 86. (Continued)

Organ and Diagnosis	Compound	Sulfone						Sulfide						Sulfoxide						Control	
	Dosage Group	High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose			
	Group[a]	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
	Number in Group	4	1	5	5	2	2	11	1	3	3	3	3	3	3	3	3	4	3	3	3
*Tonsil, lymphoid hyperplasia																		1			
*Thymus, lymphoid depletion		1		2				3		1	1			1		1					
*Thymus, atrophy		1		2				3	1	1						1					
Thymus, ectopic endocrine tissue										1											
*Thymus, medullary lymphocytes, hyperplasia																		1			
Eye, periorbital tissue, edema, focal		1																			
Periorbital tissue, mineralization		1																			
*Stomach, gastric glands, degeneration and/or necrosis		1						1	1				1								
*Stomach, lamina propria and surface epithelium, vacuolization				1																	
*Stomach, mucosa, degeneration with vacuolation, diffuse		1						4						1		1					
*Stomach, superficial epithelium, degeneration and necrosis								1													
*Stomach, gastric glands, dilitation								1													
*Stomach, mucosa, loss of surface epithelium								1													
Stomach, submucosa, gastritis, chronic, multifocal, parasitic											1										
Stomach, fundic region, mucosa, congestion															1						
Stomach, fundus, mucosa, lymphocyte and plasma cell infiltrate, diffuse																		1			
Stomach, mucosa, gastritis, chronic																				1	
Cecum, typhlitis, neutrophilic, diffuse		1																			

TABLE 86. (Continued)

Organ and Diagnosis	Compound	Sulfone						Sulfide						Sulfoxide						Control	
	Dosage Group	High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose			
	Group[a]	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
	Number in Group	4	1	5	5	2	2	11	1	3	3	3	3	3	3	3	3	4	3	3	3
Cecum, mucosa, typhlitis, suppurative, focal								1													
*Duodenum, crypt epithelium, vacuolar degeneration	1							2				1									
*Duodenum, crypt epithelium, vacuolization								1													
*Duodenum, lamina propria, cellular necrosis								1													
*Duodenum, lamina propria, congestion and/or hemorrhage								2													
Duodenum, submucosa, ectopic pancreas																			1		
*Jejunum, mucosal degeneration	1																				
*Jejunum, crypt epithelium, vacuolar degeneration	1							4	1												
*Jejunum, crypt epithelium, vacuolization					1																
*Ileum, mucosal degeneration	1							1								1					
*Ileum, crypt epithelium, vacuolization					1																
*Ileum, crypt epithelium, vacuolar degeneration								1	1												
*Colon, mucosa, degeneration	1													1							
*Colon, cell cycle alteration	1																				
Colon, congestion								1													
*Colon, surface epithelium, degeneration								1													
Colon, colitis, catarrhal, with erosions																1					
Colon, submucosa, colitis, suppurative with abscessation																			1		

TABLE 86. (Continued)

Organ and Diagnosis	Compound	Sulfone						Sulfide						Sulfoxide						Control	
	Dosage Group	High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose			
	Group[a]	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
	Number in Group	4	1	5	5	2	2	11	1	3	3	3	3	3	3	3	3	4	3	3	3
Colon, submucosa, colitis, granulomatous and eosinophilic																			1		
Colon, serosa, necrotizing granuloma, focal																			1		
*Rectum, increased mitotic activity								1													
Rectum, tunica muscularis, fibrosis, focal								1													
Rectum, tunica muscularis, hemorrhage, focal								1													
Bone marrow, hemopoietic tissue, hypoplasia	2							2													
Bone marrow, eosinophil hyperplasia											1							1			
Bone marrow, neutrophil hyperplasia														2							
*Bone marrow, lymphoreticular hyperplasia																		1			
Tongue, lymphoid infiltrate, focal		1																			
Heart, atrium, subepicardial area, lymphocytic infiltrate, focal				1																	
Heart, myocardium, lymphocytic infiltrate, focal or multifocal				2					1				1			1		1	1		2
Heart, left ventricle, subendocardial area, lymphocytic infiltrate, focal												1									
Heart, right ventricle, sarcosporidial cyst, focal																					1
Aorta, arteriosclerosis, focal								1													
Large abdominal vein, thrombosis												1									
Lateral ventricle, choroid plexus, lymphocytic infiltrate, focal																		1			

TABLE 86. (Continued)

Organ and Diagnosis	Compound	Sulfone						Sulfide						Sulfoxide						Control	
	Dosage Group	High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose			
	Group(a)	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
	Number in Group	4	1	5	5	2	2	11	1	3	3	3	3	3	3	3	3	4	3	3	3
Pancreas, ectopic spleen, focal				1	1																
*Pancreas, zymogen granule depletion, diffuse								1													
Pancreas, acinar tissue, atrophy, multifocal																		1			
Pancreas, lymphocytic infiltrate, focal or multifocal																		1			1
Diaphragm, serositis, chronic					1																
Diaphragm, lymphocytic infiltrate, focal										1											
Diaphragm, subserosal area, chronic inflammation														1							
Esophagus, lamina propria, lymphoid infiltrate, multifocal						1												1			
Esophagus, epithelium, degeneration					1						1										
Ovary, follicular cyst				1				1													
Ovary, paraovarian cyst						1															
Ovary, corpus luteum, hemorrhage														1							
Uterus, endometrium, lymphocytic infiltrate																		1			
Testis, orchitis, lymphocytic, focal																1					
Epididymis, ectopic endocrine tissue, focal																	1				
Epididymis and paraepididymal connective tissue, lymphoid nodules, multifocal																		1			
Epididymis, cystic duct, focal																					1
Epididymis, ductal adenoma																					1

TABLE 86. (Continued)

Organ and Diagnosis	Compound	Sulfone						Sulfide						Sulfoxide						Control	
	Dosage Group	High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose			
	Group(a)	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
	Number in Group	4	1	5	5	2	2	11	1	3	3	3	3	3	3	3	3	4	3	3	3
Prostate gland, lymphocytic infiltrate, multifocal																		1			
Tunica albuginea, lymphoid nodule																		1			
Ear, otitis, externa, nonsuppurative, focal							1														
Salivary gland, duct, inspissated secretion with mineralization, focal				1																	
Salivary gland, lymphocytic infiltrate, multifocal or focal				1		1				1		3	1		2		2	1		2	2
Muscle, myositis, focal, parasitic							1														
Pituitary gland, pars distalis, mineralization, focal								1													
Pituitary gland, pars intermedia, cyst																				1	
Cerebrum, lipofusin pigment deposition, multifocal														1							
Cerebrum, compression atrophy, focal														1							
Sciatic nerve, axonal degeneration with lymphocytic infiltrate, focal											1										
Mesentery, granulomas, multiple, parasitic											1										
Mesentery, pyogranuloma, focal																			1		
Skin and/or subcutaneous tissue of the elbow, fibrosis, focal								1		2											
Skin and subcutaneous tissue, hemorrhage, focal										1											

TABLE 86. (Continued)

Organ and Diagnosis	Compound	Sulfone						Sulfide						Sulfoxide						Control	
	Dosage Group	High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose		High Dose		Intermediate Dose		Low Dose			
	Group(a)	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
	Number in Group	4	1	5	5	2	2	11	1	3	3	3	3	3	3	3	3	4	3	3	3
Lip, glands of the lamina propria, lymphoid infiltrate, focal										1											
Lip, submucosal glands, lymphocytic infiltrate, multifocal												1									

(a) A = acute, R = recovery.

* Compound-related lesion.

were usually affected in the involved animals. The one exception occurred in an animal which had been treated with 5 mg/kg of sulfone. The lesion in this animal occurred in the mandibular lymph node and was characterized by a large discrete nodular proliferation of lymphoreticular cells as described previously. There was a moderate amount of lymphoid necrosis in this lesion and there was compression of the surrounding lymphoid elements. This was a single focus involving one large nodule with an adjacent small nodule in the lymph node. There were no other such lesions observed in lymph nodes, spleen, or other lymphoid tissue in this animal. Lymphoid hyperplasia in the tonsil, and in some cases the thymus, was also observed in involved animals. These lesions were observed in four animals, at least one of which was from a high, intermediate, or low dosage group from each of the three compounds. The diffuse proliferations were observed in animals treated with 80 and 40 mg/kg of sulfide and 30 mg/kg of sulfone, all of which were moribund and terminated on day 9 or 10. The nodular lesions which involved lymph nodes, spleen, and bone marrow were observed in a single animal which was treated with 5 mg/kg of sulfoxide and terminated on day 15. The solitary nodular lesion was observed in an animal treated with 5 mg/kg of sulfone and terminated on day 15. The lesions in these animals were diagnosed as reactive hyperplasia, with the exception of the single nodular lesion in the animal given 5 mg/kg of sulfone which was diagnosed as nodular lymphoma, focal. Since the lesions were early in their development, the differentiation between hyperplasia and neoplasia was difficult; however, the nature of the proliferations and the pattern of growth were considered to be consistent with the diagnoses given. In addition to the lesions described above, lymphoid depletion of lymph nodes or tonsils involving germinal centers specifically or a more generalized lymphoid depletion was observed in three animals from both the high and intermediate dose groups of sulfone and two animals from the high dose groups of both sulfide and sulfoxide. These animals were all terminated on day 15. Thymic atrophy and depletion were noted in animals from both the high and intermediate dose groups of all three compounds. Generalized lymphoid hyperplasia was also observed in lymph nodes of one animal from the low dose group of sulfoxide.

Liver lesions were common in animals treated at the higher dosage levels. These occurred in all three compounds but were most prominent in the animals treated with the highest dosage levels of sulfide with fewer numbers involved in the animals given sulfoxide and only minor involvement of animals treated with sulfone. The lesions consisted of vacuolization of hepatocytes with or without hepatocyte degeneration or necrosis. The less severe lesions involved vacuolization of hepatocytes which was either diffuse or of centrilobular distribution. The more severe lesions were characterized by a vacuolar or granulovacuolar cytoplasmic change in hepatocytes which was most prominent in centrilobular areas, but which in some instances, extended diffusely throughout the liver parenchyma. In some instances, sinusoids were irregularly widened and there was evidence of degeneration and necrosis in individual hepatocytes, giving the impression that there was an absolute loss of hepatocytes, especially in centrilobular areas. In one animal treated at the lowest dosage level of sulfoxide, megalocytosis was observed in hepatocytes predominantly in centrilobular areas. While the hepatic lesions were most prominent in the animals from higher dosage levels, they were observed in intermediate and low dose groups, especially in animals treated with sulfoxide. They were also observed in animals that had gone through the 2-week recovery period, as well as those terminated immediately following treatment.

Renal lesions were also observed in several animals involving primarily those treated at the highest dosage levels in all three dosage groups. The lesions consisted of the deposition of brown granular pigment in the basal portion of the proximal tubular epithelium; this pigment was interpreted to be lipofuscin pigment. Vacuolization of proximal tubular epithelium was also observed in three animals, one each from the high and intermediate dosage groups of sulfide and one from the highest dosage group of sulfoxide. Glomerular sclerosis was observed in two animals treated at the high dosage level of sulfide; however, it was impossible to determine whether this lesion was associated with compound administration.

Lesions in the adrenal cortices were prominent in animals treated with high or intermediate dosage levels from all three compounds.

These lesions consisted of cortical hyperplasia, usually with areas of hemorrhage and congestion. In some instances, vacuolization or vacuolar degeneration was observed in cells of the zona fasciculata and these were nearly always associated with the areas of hemorrhage.

Thyroid glands of several animals contained changes which consisted of follicular cell hyperplasia. A moderately large focus of follicular degeneration with the follicular cell hyperplasia and loss of thyroglobulin occurred in one animal from the high dose group of sulfide.

Changes were observed in the digestive tract of several animals, being most common in those treated with high dose levels of sulfide. Lesions in the stomach consisted of vacuolar changes or vacuolization with degeneration in the superficial lamina propria and in some cases, involved the superficial epithelium. This was usually a diffuse lesion and present only in animals treated at the higher dosage levels. Degeneration and necrosis was also observed in the gastric glands of several animals and in some instances, this predominantly involved the parietal epithelial cells. Changes throughout the small intestine of animals from all three compounds were quite consistent and were observed most prominently in the animals given the high dose levels of sulfide. These lesions consisted of vacuolization or vacuolar degeneration in the deepest layers of the crypts. In some animals this appeared to involve the Paneth cells primarily, while in others it was not possible to determine if a specific cell type was involved. In several instances, this change was quite mild and difficult to discern from the vacuolar appearance that is normally present in cells of this area from control monkeys. There was evidence of changes involving cell turnover or cell migration in several animals and this change resulted in mucosal degeneration characterized by shortening and fusing of villi and extension of mitotic activity further up the crypt than is normally the case. These changes were seen more commonly in the ileum but were also present in the jejunum in one animal treated with a high dose level of sulfone. Changes in the colon and rectum were relatively infrequent and consisted of mucosal degeneration, cell cycle alteration, degeneration of surface

epithelium, or increased mitotic activity, usually occurring in single animals at the high or intermediate dose levels of these compounds. The only other lesion that was considered to be drug induced was a diffuse depletion of zymogen granules observed in the pancreas of one animal treated at the highest dosage level of sulfide.

Other lesions observed in these animals were considered to be spontaneous due either to the nature of the lesions, their common occurrence in "normal" rhesus monkeys, or the occurrence of the change in control groups with a frequency similar to that observed in treated animals.

ELECTROCARDIOGRAMS

Visual inspection of the electrocardiograms by Dr. Robert Hamlin revealed numerous subtle but insignificant changes. Monkey 725BF appeared to develop notching of QRS on June 2, 1978, while monkey 776AM had right bundle branch block (RBBB). Many monkeys had slight intraventricular conduction disturbance that seemed to change unsystematically. Genesis and interpretation of these disturbances are equivocal, but they are probably of no physiological or clinical significance.

PHARMACOKINETICS

METHODS

Rats

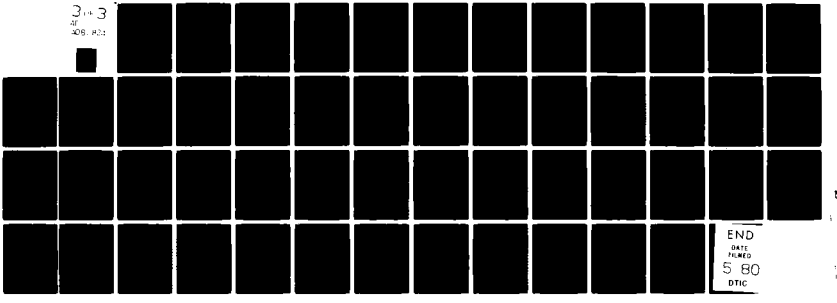
Preweighed animals (rats) were randomized in eight groups, each experimental group having six animals per sex. The groups designated 1, 2, 3, 4, 5, 6, 7, and 8 corresponded to those in which samples were taken at 0.5 hours, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 72 hours, and 168 hours, respectively. Rats in all groups were fasted overnight before dosing. Each animal was given 20 µci of ^{14}C (benzene ring)-labeled chemical in 0.2 ml of corn oil via oral gavage. Tail markings were used to identify animals in each group.

Each animal was anesthetized with ether and blood was collected by cardiac puncture in 3 ml B&D EDTA containing tubes according to the designated time period (0.5 hours through 7 days). At least 2 ml of blood were collected from each animal and kept frozen. Animals were terminated following blood collection.

Duplicate blood samples (0.1 ml) were oxidized in a Tri-Carb Packard oxidizer. The instrument was set for 1 minute which was adequate time to completely burn the sample. The instrument pump was set to deliver a 5 ml Carb-Sorb and 10 ml Liquid Scintillant cocktail. A Searle Analytic Counter was used to count the ^{14}C in the samples. Average ^{14}C counts were calculated as DPM/1 ml of blood.

After dosing, all animals from Group 8 were individually caged in metabolism cages for 7 days. Urine and fecal samples were collected daily for 7 days for each 24-hour period. Feces were collected once for each 24-hour period. Total urine volume was recorded for 24 hours and total fecal volumes were recorded as grams per 24-hour period. Animals from this group were anesthetized with ether on day 7 after the final urine and fecal samples were collected. Blood samples obtained by cardiac puncture were then collected in EDTA containing tubes after which the animals were terminated.

AD-A082 824 BATTELLE COLUMBUS LABS OH F/G 6/20
MAMMALIAN TOXICOLOGICAL EVALUATION OF P-CHLOROPHENYL METHYL SUL--ETC(U)
JUL 79 D C THAKE, D MAYS, P LEBER, D METCALF DAMD17-77-C-7038
UNCLASSIFIED NL



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Specimens were taken from 24-hour urine samples and directly added to 10 ml of aqueous counting scintillant and counted in a Searle Analytic Counter. Total ^{14}C radioactivity was calculated according to total 24-hour urine volume and reported as percent of dose excreted.

Two-tenths milliliter of the homogeneous slurry sample was Oxidizer in a Tri-Carb Packard oxidizer using conditions as described previously and the ^{14}C radioactivity was counted in a Searle Analytic Counter. Total radioactivity was calculated according to the total volume of homogeneous slurry and reported as percent dose excreted.

Rhesus Monkeys

Each experimental group consisted of four male monkeys. Monkeys were kept in restraint chairs for 7 days and fasted overnight prior to administration of the test compounds. Acetone (0.1 ml) was used to dissolve 12.5 mg ^{14}C-labeled chemical and 112.5 mg cold chemical and the volume was extended to 5 ml with corn oil. One ml of corn oil was given to each monkey by oral gavage. Each dose contained a total of 25 mg chemical including 100 µci of ^{14}C-labeled chemical.

Urine and fecal samples were collected at 24-hour intervals on days 1 through 7.

Blood samples were collected in EDTA containing tubes at 0.5 hours, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 72 hours, 7 days, 14 days, and 28 days. Procedures for counting radioactivity and calculation of results were identical to those described above for rats.

RESULTS

Several pharmacokinetic parameters were found to be the same for the different chemicals and are listed below (also see Figures 17-28):

1. In rats, the disappearance of ^{14}C was biphasic with the first phase having a half-life ($t_{1/2}$) of 9 to 12 hours and phase 2 a $t_{1/2}$ of 1.6 to 3.4 days.

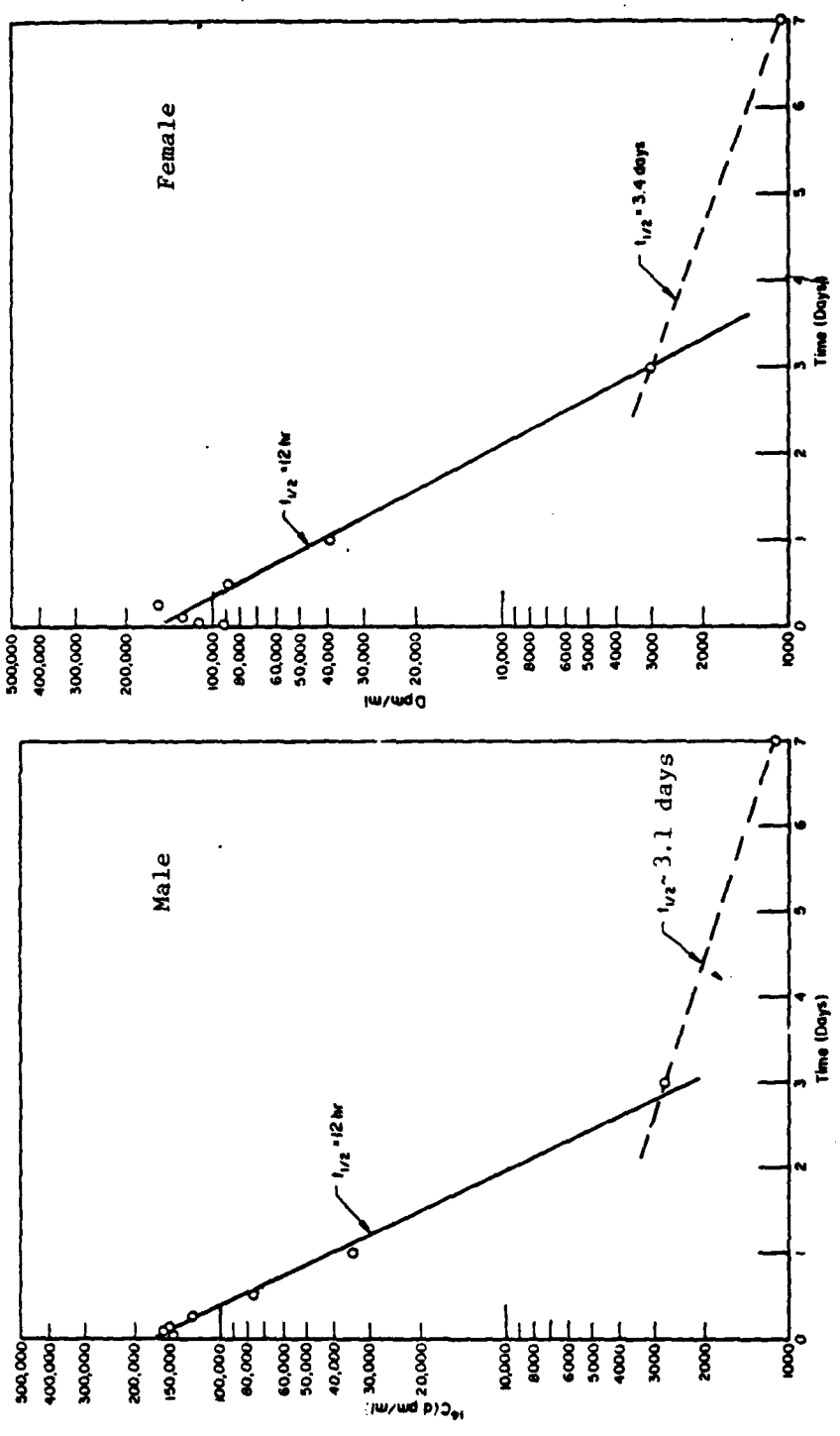


FIGURE 17. BLOOD LEVELS OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFIDE TO RATS

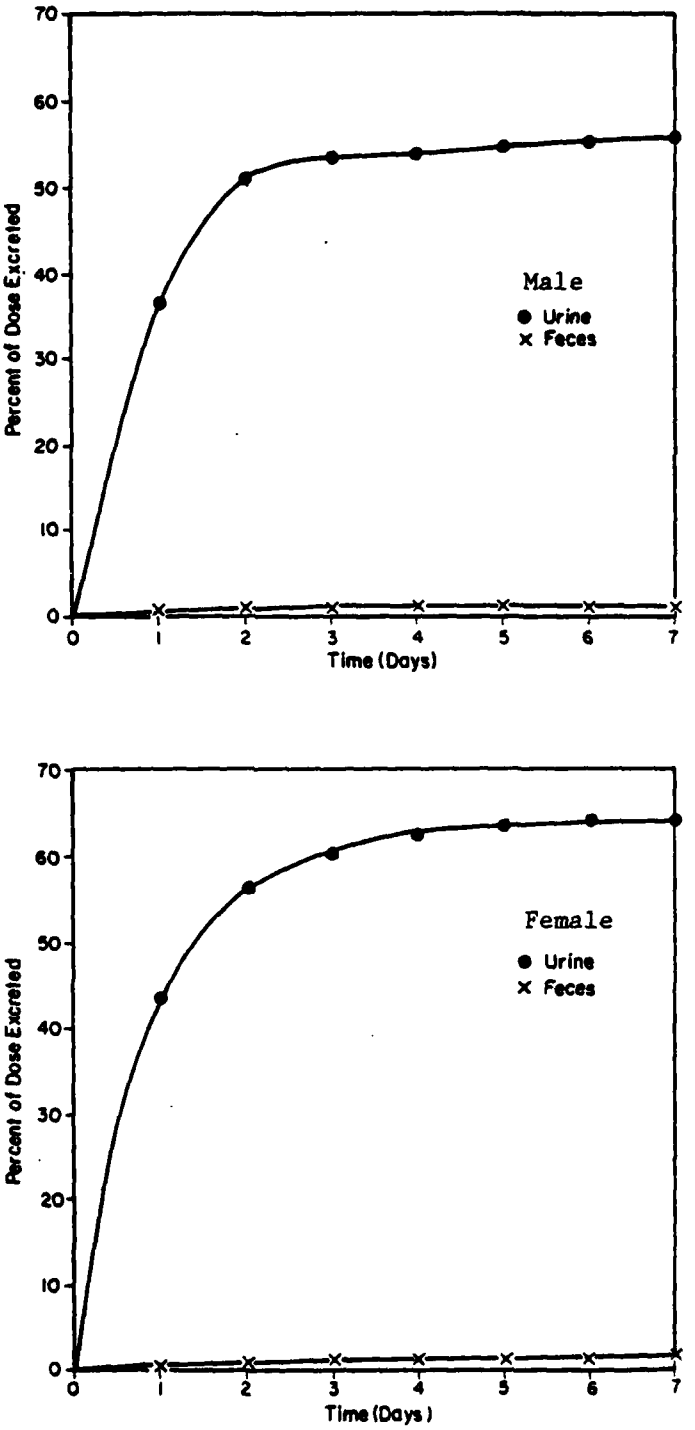


FIGURE 18. EXCRETION OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFIDE TO RATS

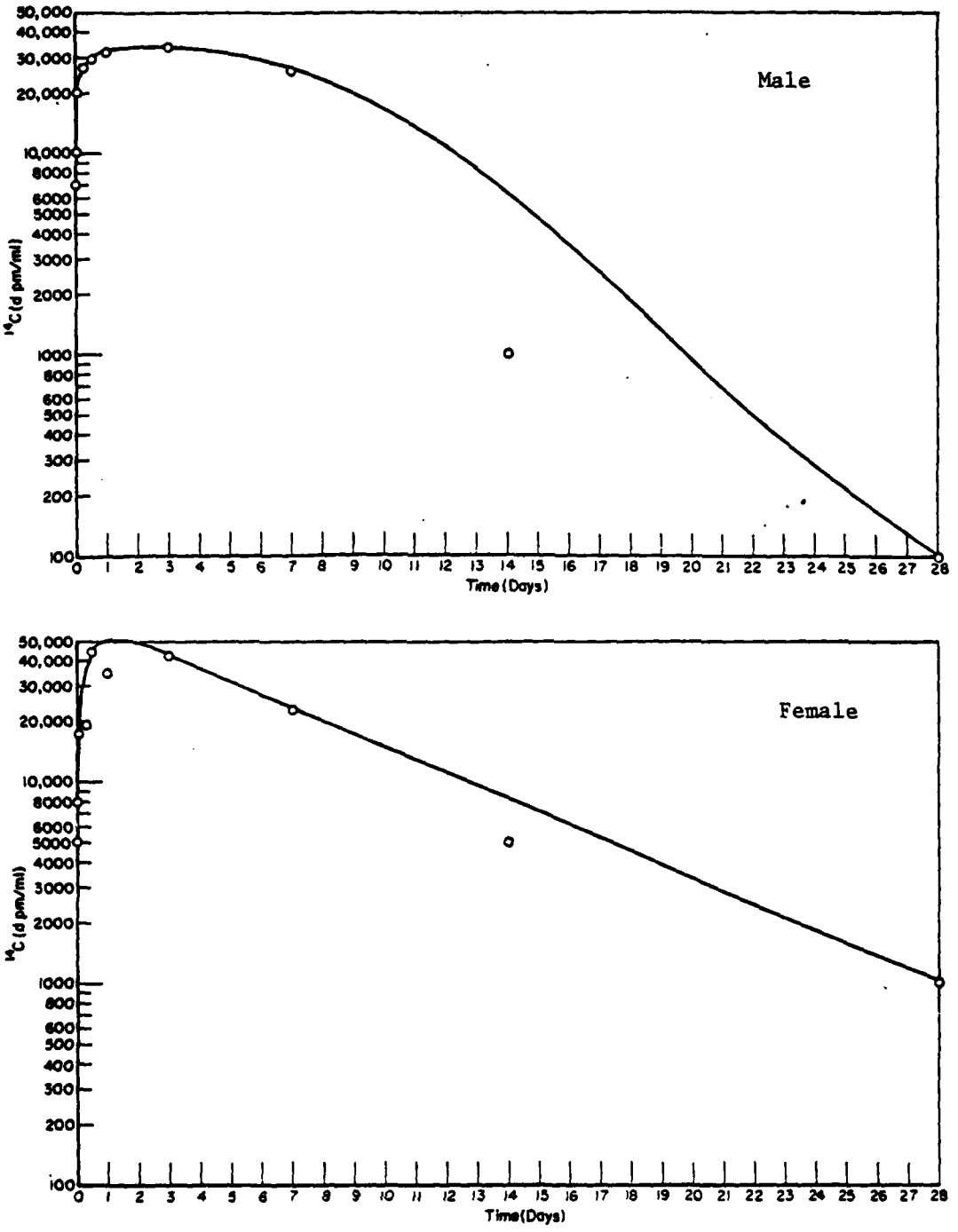
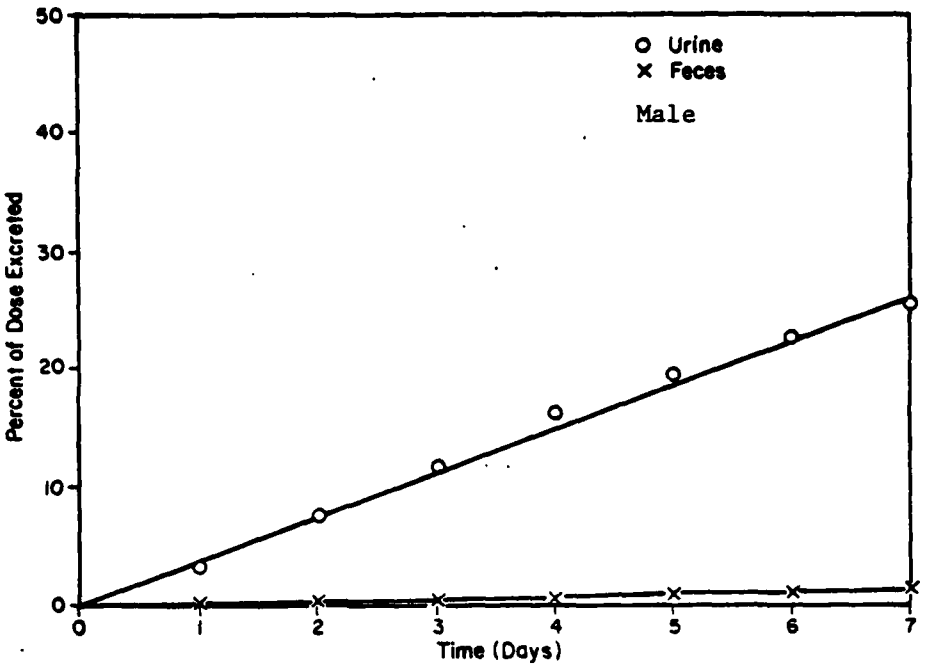


FIGURE 19. BLOOD LEVELS OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFIDE TO RHESUS MONKEYS



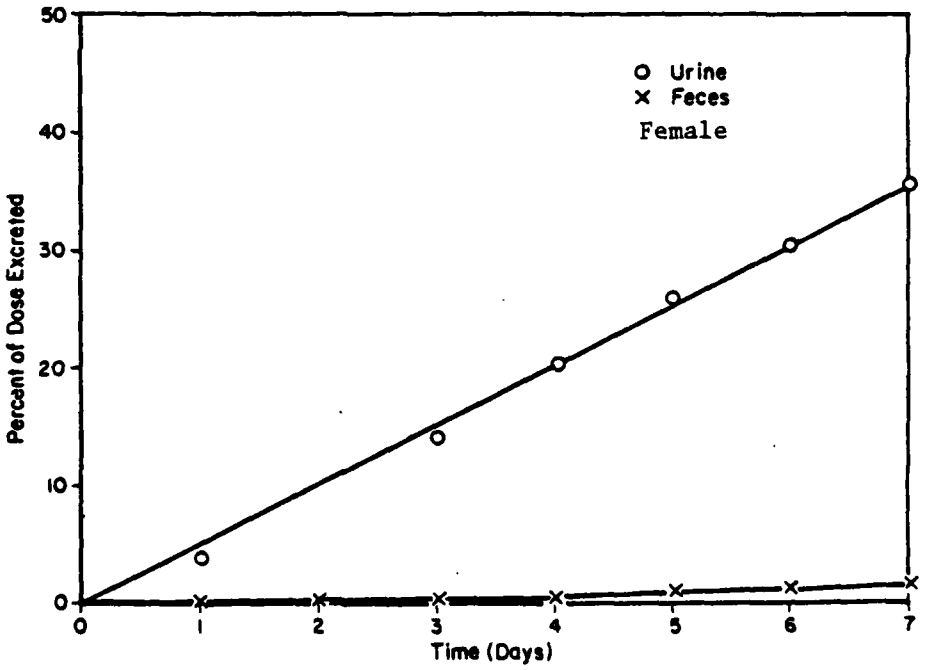


FIGURE 20. EXCRETION OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFIDE TO RHESUS MONKEYS

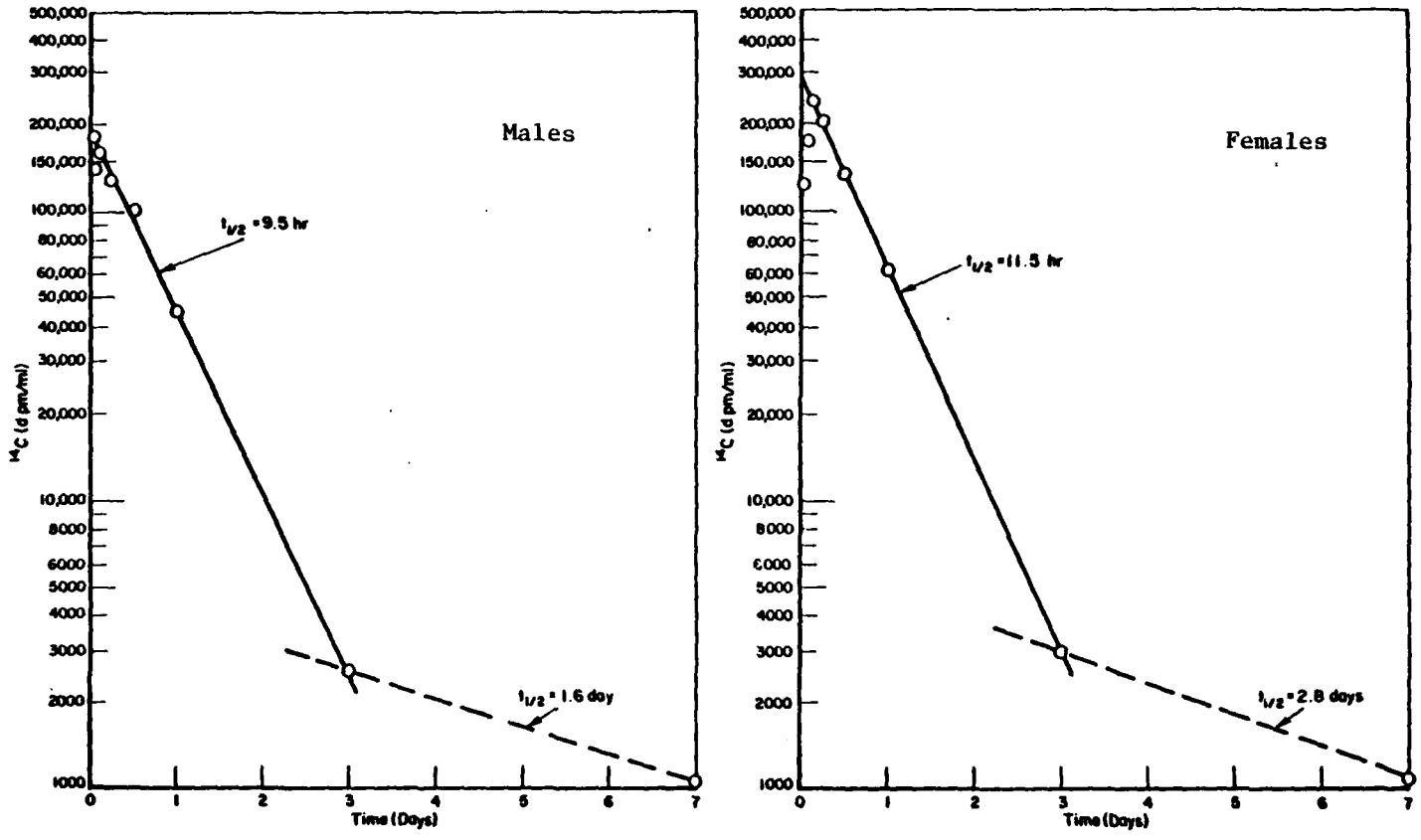
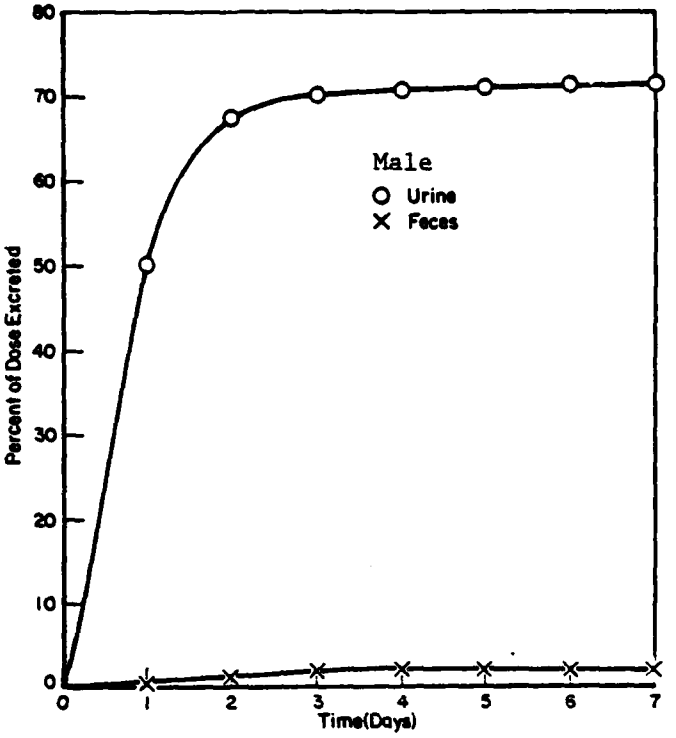


FIGURE 21. BLOOD LEVELS OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFONE TO RATS



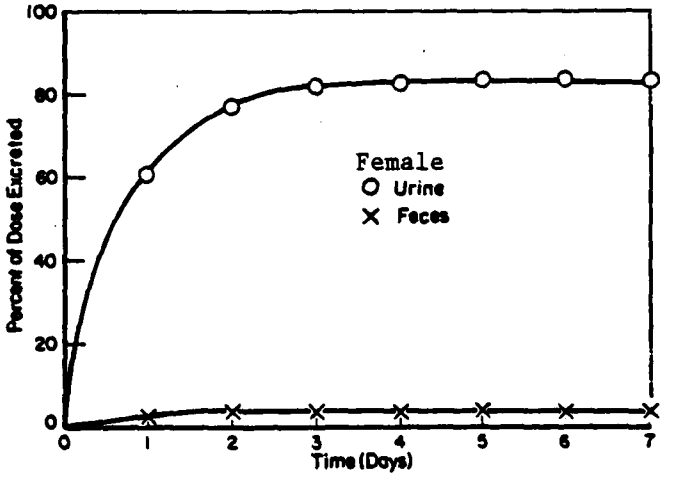


FIGURE 22. EXCRETION OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFONE TO RATS

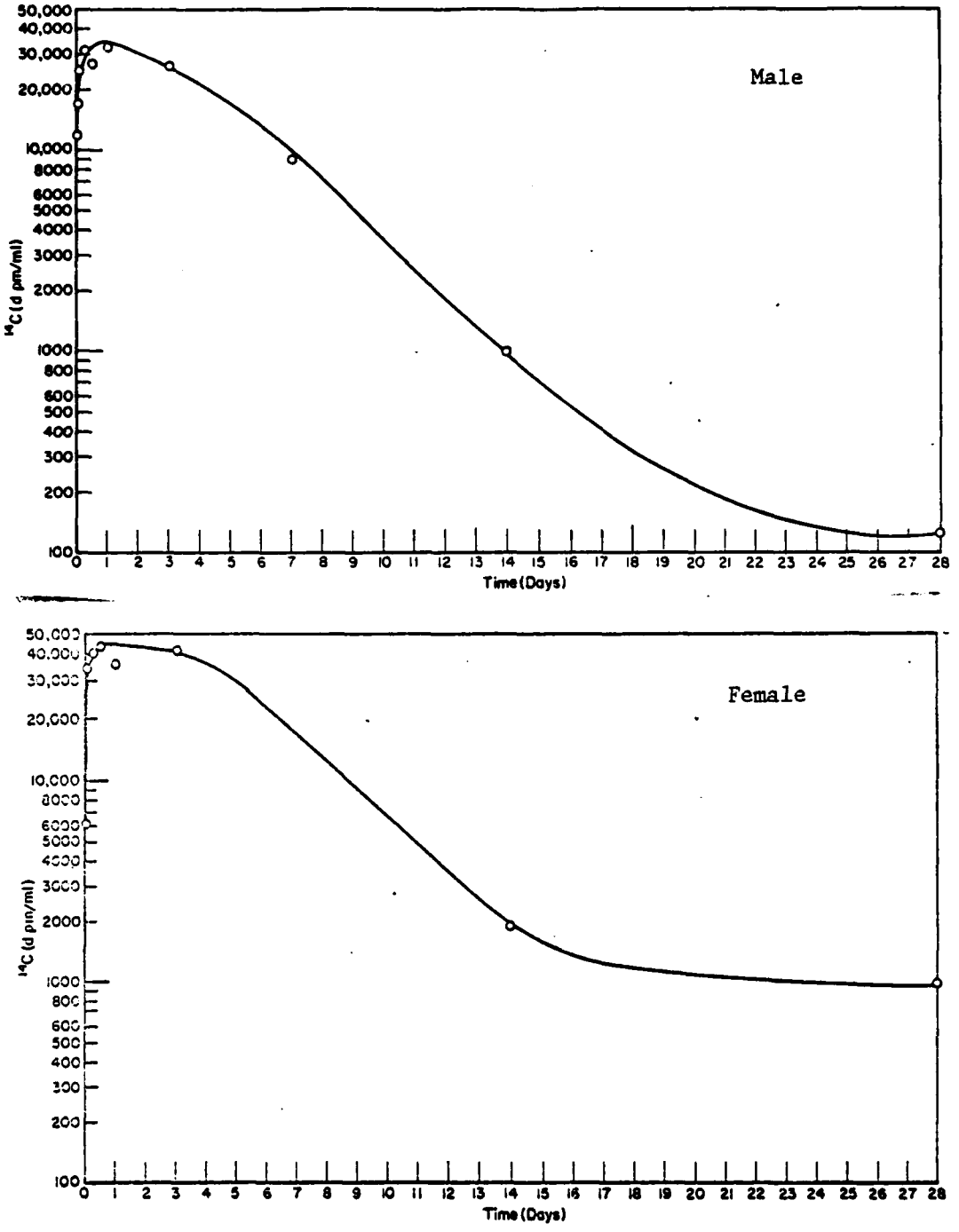
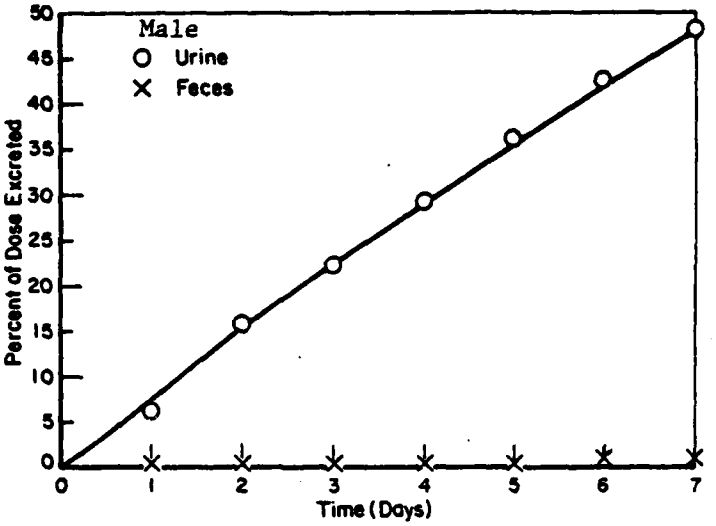


FIGURE 23. BLOOD LEVELS OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFONE TO RHESUS MONKEYS



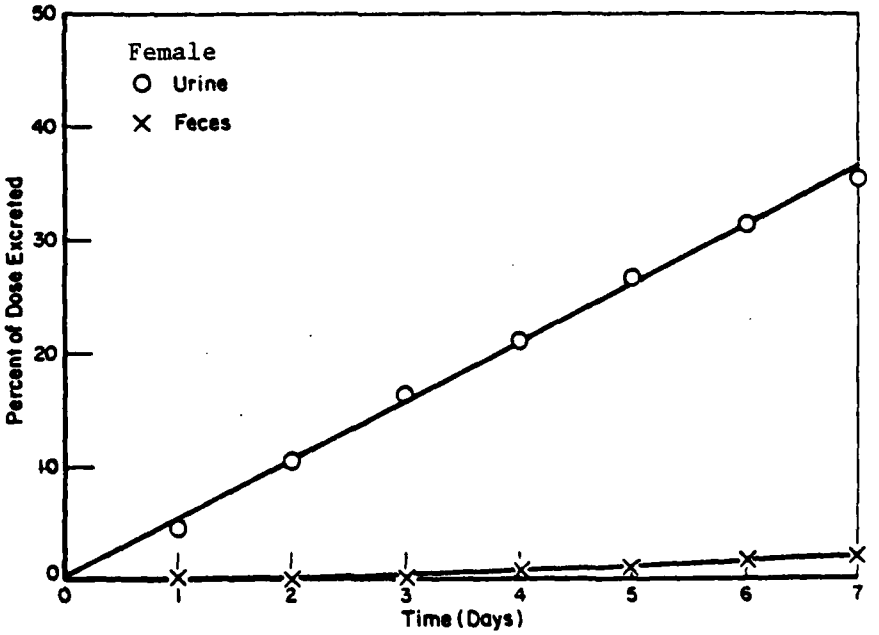


FIGURE 24. EXCRETION OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFONE TO RHESUS MONKEYS

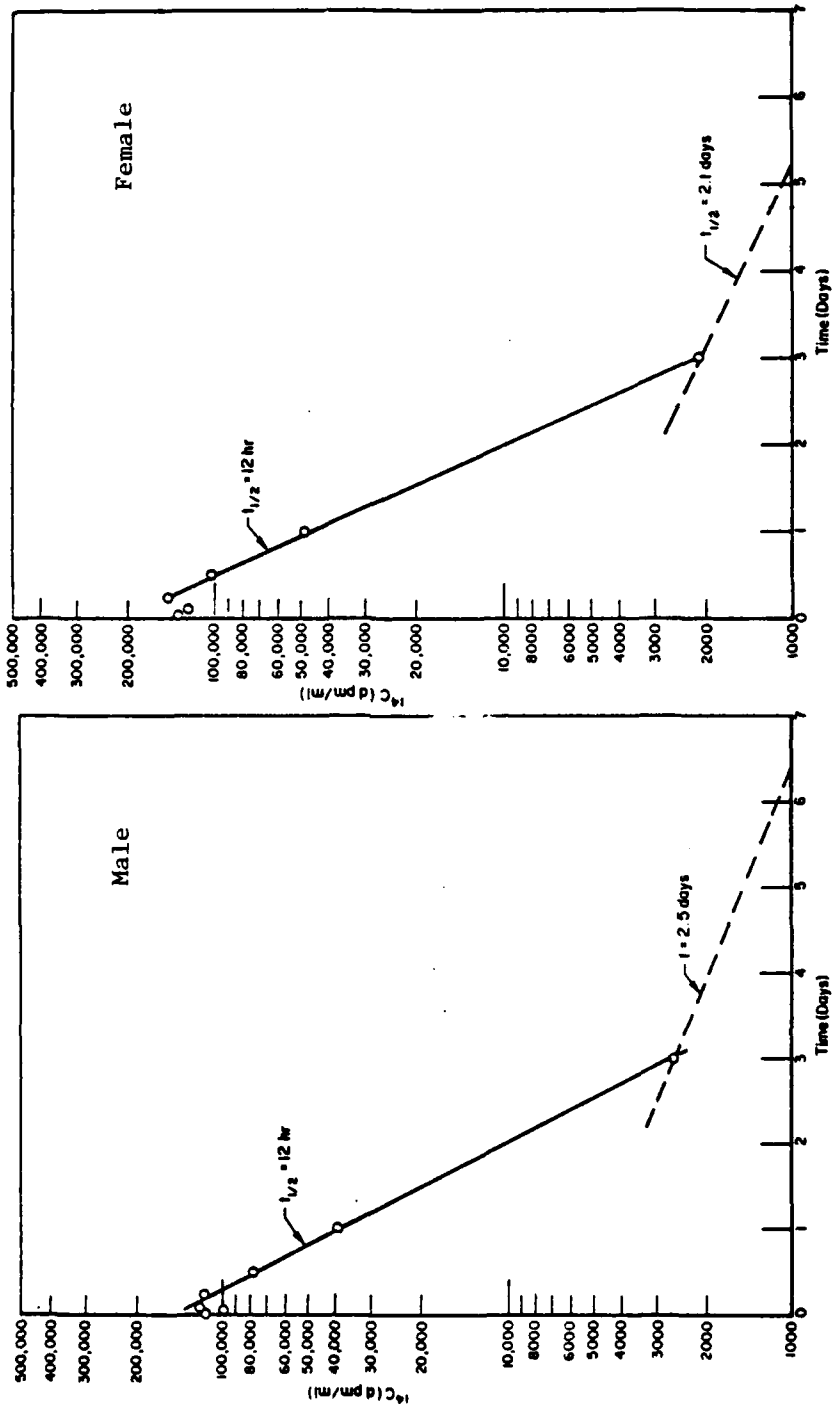


FIGURE 25. BLOOD LEVELS OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFOXIDE TO RATS

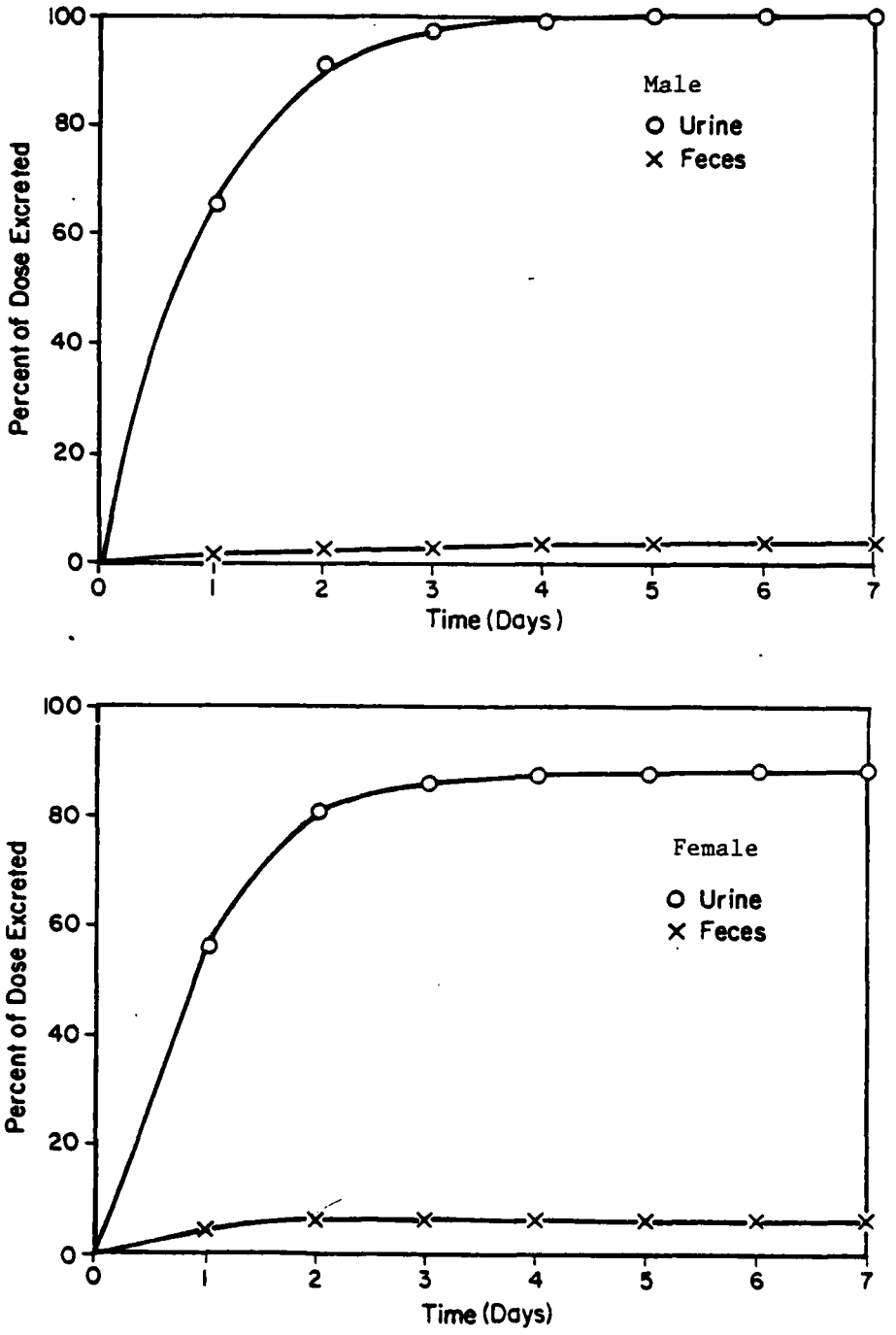


FIGURE 26. EXCRETION OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFOXIDE TO RATS

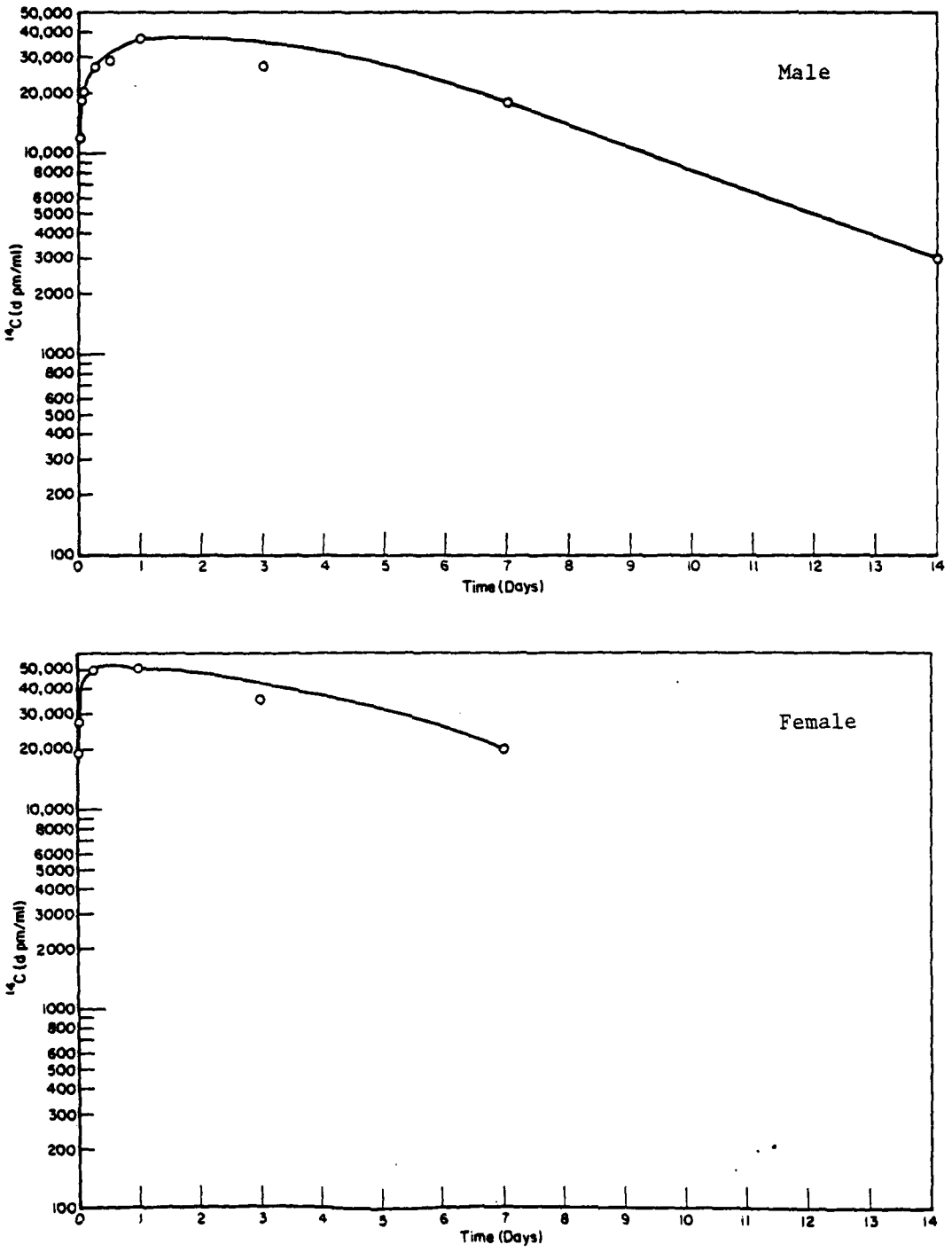


FIGURE 27. BLOOD LEVELS OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFOXIDE TO RHESUS MONKEYS

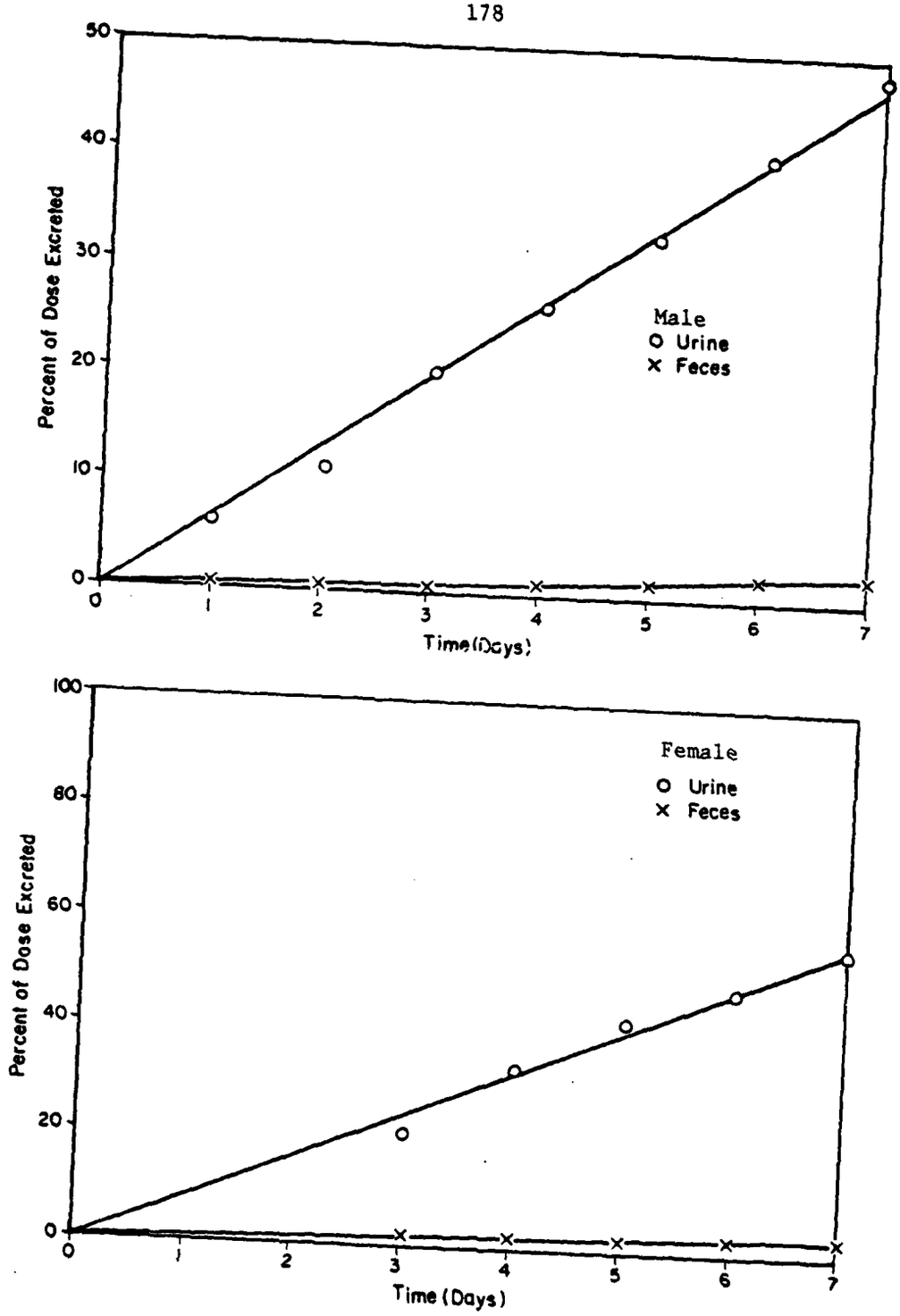


FIGURE 28. EXCRETION OF ^{14}C FOLLOWING ADMINISTRATION OF ^{14}C p-CHLOROPHENYL METHYL SULFOXIDE TO RHESUS MONKEYS

2. The elimination kinetics of ^{14}C in the urine was different between species. Rats excreted the largest percentage the first day (35-65%) and 50 to 90% by the end of the second day, whereas rhesus monkeys excreted constant percentages for 7 days. The "zero order" kinetics for the monkeys indicates that the renal elimination is saturable, as opposed to the rat situation which is not a saturable process. The slight difference in doses (2.5 mg/kg for rats and approximately 6 mg/kg for monkeys) for the ^{14}C chemicals is not likely responsible for the kinetic differences.
3. The fecal elimination of ^{14}C was 8% or less of the total dose administered for both species, and therefore accounts for only a small proportion of the total elimination.

The kinetics of ^{14}C disappearance in the blood of rhesus monkeys was followed for up to 28 days. Male levels of ^{14}C declined more rapidly than for females for both sulfide- and sulfone-treated monkeys. No significant differences are apparent for the blood curves of the different chemicals.

METABOLISM

METHODS

Rats

Urine collected from each rat over a 24-hour period was pooled for these studies. Since pharmacokinetic data showed that more than 50% of the dose was excreted in 24 hours, urine for metabolism studies was obtained during the first 24 hours following administration of the test compounds.

A 10 ml sample of pooled 24-hour urine was used to obtain a 0.1 ml specimen for ^{14}C radioactivity counting.

The 10 ml urine sample was adjusted to pH 1.9 and extracted with two 10 ml volumes of chloroform. Total radioactivity in the 10 ml urine sample was designated as 100%. The 0.1 ml chloroform extract was counted for radioactivity and percent recovery at pH 1.9 was reported.

The low recovery (approximately 6 to 10%) indicated that most of the chemical was conjugated; therefore, the urine sample was adjusted to pH 5.5 and 1.0 ml of glusulase sulfatase enzyme was added and the samples were incubated for 24 hours at 37 C. After enzymatic hydrolysis, the urine samples were again extracted with two 10 ml volumes of chloroform. One-tenth ml of the chloroform extract was counted for ^{14}C radioactivity and reported as percent recovery of ^{14}C radioactivity after hydrolysis. There remained 10 to 15% of the radioactivity which was not recovered.

Rhesus Monkeys

Urine samples from rhesus monkeys were treated in a manner identical to those of rats except that individual samples were used rather than pooled samples as was the case in rats. Instrument specifications for gas chromatographic studies are shown below.

Instrument:	Varian 2100 Aerograph
Column:	6' x 1/4 O D glass column packed with 10% FFAP on 8/100 mesh chromosorb WAW
Instrument Condition:	
Injection temperature	230 C
Detector temperature	270 C
Column temperature + program	150 to 230 C/4 C minute
N_2 carrier flow rate	40 ml/minute.

As described above, enzyme hydrolyzed (pH 5.5) chloroform extracts (pH 1.9) were subjected to gas chromatography. The chloroform extract was evaporated to dryness and dissolved in 0.2 ml of acetone. Gas chromatographic analyses were performed using conditions described above. These samples were also submitted for mass spectral analysis but the data were not conclusive.

Thin layer chromatography and mass spectroscopy studies were performed on only the unhydrolyzed fractions of the $CHCl_3$ extracts of rat urine. Undesirable impurities were removed by using thin layer chromatography which was performed using a Packard Radiochromatograph Scanner. Thin layer plates were obtained from Quantum Industries, type LQGD in 5 x 20 sizes. Thin layer plates were spotted with chloroform extract containing ^{14}C-labeled compound. These plates were developed in toluene and ethyl acetate using a 1:1 ratio.

The developed thin layer plates were subjected to radiochromatogram scanning and two major peaks or spots were observed. One of the peaks correlated with the sulfone standard.

This spot was scraped from TLC plates and subjected to mass spectroscopy to confirm the presence of sulfone.

Interconversion

Interconversions of sulfide, sulfoxide, and sulfone *in vivo* and *in vitro* were investigated further in young adult Fischer 344 male rats by gas chromatographic mass spectrophotometric analysis.

Urine samples from treated and untreated rats were collected for a 24-hour period following a single administration of 50 mg/kg sulfide, sulfoxide, or sulfone dissolved in corn oil or corn oil alone. Equal volumes of pooled urine from treated and untreated animals were extracted twice with 10-ml volumes of chloroform. Aliquots of urine from untreated animals were spiked with either sulfide, sulfoxide, or sulfone; allowed to stand at room temperature for 24 hours; and extracted twice with 10-ml volumes of chloroform. Each of the combined extracts of urine (treated, untreated, and spiked) were concentrated from a volume of 20 ml to a volume of approximately 100 µl prior to GC-MS analysis.

The GC-MS analyses were run using a Finnigan Model 4000 Gas Chromatograph-Mass Spectrometer interfaced to an Incos Model 2300 data system. The GC column was 3% OV-17. The temperature program was from 70 C to 270 C at 8 degrees per minute. The mass spectra were acquired under electron impact conditions. The electron energy was 70 EV; the electron emission current was 300 µa; and the source housing pressure was 3×10^{-6} torr (with helium). The electron multiplier was operated at 1400 volts (gain x 1×10^{-4}) and the preamplifier at a gain of 10^7 volts per amp. The chamber temperature was 210 C, the jet separator 250 C, and the transfer line 275 C. Ion optics tuning and mass calibration were done prior to the analyses. The injected sample size was 1.0 µl for the spiked urine samples and 3 µl for the urine blank and treated rat urine samples.

RESULTS

Rats and Monkeys

Table 87 presents data on the extraction of ^{14}C from urines from the chemically-treated test animals. The urines were extracted at the indicated pHs before and after enzymatic hydrolysis with chloroform.

The "before hydrolysis" data indicate that rats are converting the chemicals to water-soluble compounds to a greater extent than monkeys, which may have toxicological significance since rats tolerate the chemicals

TABLE 87. EXTRACTION OF ^{14}C FROM URINE OF ^{14}C-p-CHLOROPHENYL METHYL SULFIDE-, SULFONE-, AND SULFOXIDE-TREATED RATS AND RHESUS MONKEYS (PERCENT CONTAINED IN URINE)

Group		Before Hydrolysis: Extract c̄ 2 x 10 ml $CHCl_3$ pH 1.9	After Hydrolysis*: Extract c̄ 2 x 10 ml $CHCl_3$ pH 5.5, then pH 1.9	
SULFIDE	Rats			
	M	5.7%	45.38%	8.3%
	F	5.25%	42.19%	6.25%
	Rhesus Monkeys			
	M	21.78%	48.86%	3.20%
	F	26.10%	39.96%	6.86%
SULFONE	Rats			
	M	6.33%	37.99%	6.31%
	F	4.20%	37.85%	6.19%
	Rhesus Monkeys			
	M	16.53%	40.87%	6.95%
	F	15.35%	40.56%	7.67%
SULFOXIDE	Rats			
	M	6.19%	48.72%	3.84%
	F	5.5%	48.13%	7.34%
	Rhesus Monkeys			
	M	55.0%	4.0%	1.89%
	F	62.9%	6.0%	9.04%

* 1.0 ml enzyme Glusulase sulfatase added to 10 ml urine (pH 5.5) and allowed to incubate for 24 hours at 37 C temperature.

better than the monkeys. It is apparent that both species do conjugate the test chemicals or their metabolites since substantial ^{14}C is extracted after glucuronidase-sulfatase catalyzed hydrolysis. Rhesus monkeys did not conjugate the sulfoxide to a significant degree.

Tables 88-90 present data on the gas chromatographic separations of the chloroform extracts performed in Table 87. The results are summarized below:

1. Sulfide-treated animals (Table 88). Sulfide was found in all rat urines, as was sulfoxide. Sulfone presence is not certain because of the retention times which are not within the normal standard range. Smaller amounts of free sulfide were found in the rhesus urines. The presence of sulfoxide is questionable (retention times), but sulfone does appear to occur in these urines prior to hydrolysis.
2. Sulfone-treated animals (Table 89). Sulfone and sulfoxide may be found free in all urines but is questionable (retention times). Sulfide appears in all urines.
3. Sulfoxide-treated animals (Table 90). Sulfoxide was found in rat urines, as was sulfide and sulfone. Hydrolysis led to increased amounts of extractable sulfoxide in these urines. Rhesus urines contained only trace amounts of sulfoxide and no sulfide, while one specimen contained a large amount of sulfone.

There was evidence for the interconversion of the three test chemicals in rats and rhesus monkeys. However, further work was needed employing mass spectroscopy to confirm chemical identities and interconversion of these compounds.

Interconversion

The GC-MS run of blank urine disclosed three major peaks and 10-15 minor peaks. Two of the major peaks have been tentatively identified

TABLE 88. CHEMICAL IDENTIFICATION OF COMPOUND IN URINARY EXTRACTS OF ^{14}C-p-CHLOROPHENYL METHYL SULFIDE-TREATED RATS AND RHESUS MONKEYS

Treatment Group	Enzymatic Hydrolysis*	Chemical Detected in Chloroform Extract of Urines by Gas Chromatography: Sulfide, Retention Time in Minutes	Sulfide, Calculated Mass, ng/ml Urine	Sulfoxide, Retention Time in Minutes	Sulfoxide, Calculated Mass, ng/ml Urine	Sulfone, Retention Time in Minutes	Sulfone, Calculated Mass, ng/ml Urine
Rats							
M	- (pH 1.9)	9.3	32.0	23.5	9.6	30.9	16.0
M	+ (pH 5.5)	9.3	12.8	23.5	28.8	**	**
F	- (pH 1.9)	9.3	35.2	23.7	6.4	30.9	12.8
F	+ (pH 5.5)	9.4	16.0	23.6	51.2	31.2	6.4
Rhesus Monkeys							
M	- (pH 1.9)	**	**	**	**	30.0	51.2
M	+ (pH 5.5)	9.4	9.6	23.7	40.0	30.0	19.2
F	- (pH 1.9)	9.4	11.2	23.7	81.6	30.0	80.0
F	+ (pH 5.5)	9.4	14.4	24.0	67.2	**	**
STD N = 4		8.8 to 9.3	2000	23 to 23.5	2000	29.7 to 30.2	2000

* Enzyme Hydrolysis: 1 ml enzyme Glusulase sulfatase added (pH 5.5) to 10 ml urine and incubated for 24 hours at 37 C temperature--before hydrolysis and after hydrolysis.

** No peak within 1 minute of expected retention time.

TABLE 89. CHEMICAL IDENTIFICATION OF COMPOUND IN URINARY EXTRACTS OF ^{14}C-p-CHLOROPHENYL METHYL SULFOXIDE-TREATED RATS AND RHESUS MONKEYS

Treatment Group	Enzymatic Hydrolysis*	Chemical Detected in Chloroform Extract of Urines by Gas Chromatography: Sulfide — Retention Time in Minutes	Sulfide — Calculated Mass, ng/ml Urine	Sulfoxide — Retention Time in Minutes	Sulfoxide — Calculated Mass, ng/ml Urine	Sulfone — Retention Time in Minutes	Sulfone — Calculated Mass, ng/ml Urine
Rats							
M	- (pH 1.9)	11.7	52.8	29.2	12.0	38.4	17.6
M	+ (pH 5.5)	12.0	11.2	28.8	78.4	39.6	19.2
F	- (pH 1.9)	11.7	43.2	29.2	6.4	38.1	3.2
F	+ (pH 5.5)	11.7	12.8	28.5	108.8	**	**
Rhesus Monkeys							
M	- (pH 1.9)	**	**	29.0	4246.4	**	**
M	+ (pH 5.5)	**	**	29.5	10.2	36.7	40.0
F	- (pH 1.9)	**	**	23.7	96.0	29.7	6.4
F	+ (pH 5.5)	**	**	**	**	**	**
STD (1) N = 2		11.0 to 11.2		28.3	2000	38.1	2000
(2) N = 4		8.8 to 9.3		23.0 to 23.5	2000	29.7 to 30.2	2000

* Enzyme Hydrolysis: 1 ml enzyme Glusulase sulfatase added (pH 5.5) to 10 ml urine and incubated for 24 hours at 37 C temperature--before hydrolysis and after hydrolysis.

** No peak within 1 minute of expected retention time.

TABLE 90. CHEMICAL IDENTIFICATION OF COMPOUND IN URINARY EXTRACTS OF ^{14}C-p-CHLOROPHENYL METHYL SULFONE-TREATED RATS AND RHESUS MONKEYS

Treatment Group	Enzymatic Hydrolysis*	Chemical Detected in Chloroform Extract of Urines by Gas Chromatography: Sulfide — Retention Time in Minutes	Sulfide — Calculated Mass, ng/ml Urine	Sulfoxide — Retention Time in Minutes	Sulfoxide — Calculated Mass, ng/ml Urine	Sulfone — Retention Time in Minutes	Sulfone — Calculated Mass, ng/ml Urine
Rats							
M	- (pH 1.9)	9.3	4.8	24.0	5.1	29.2	9.6
M	+ (pH 5.5)	9.3	27.2	24.0	56.0	29.0	8.0
F	- (pH 1.9)	9.3	16.0	24.0	1.6	29.0	4.8
F	+ (pH 5.5)	9.6	44.8	24.2	35.2	**	**
Rhesus Monkeys							
M	- (pH 1.9)	9.3	5.4	24.0	38.4	30.2	28.8
M	+ (pH 5.5)	9.3	2.2	23.7	13.6	29.0	4.16
F***	- (pH 1.9)	9.3	2.2	24.0	1.6	30.0	15.5
F***	+ (pH 5.5)	**	**	24.2	6.4	29.2	3.2
STD N = 4		8.8 to 9.3	2000	23.0 to 23.5	2000	29.7 to 30.2	2000

* Enzymatic Hydrolysis: 1 ml enzyme Glusulase sulfatase added (pH 5.5) to 10 ml urine and incubated 24 hours at 37 C temperature--before hydrolysis and after hydrolysis.

** No peak within 1 minute of expected retention time.

*** Performed under same conditions as STD (2).

as methyl cresol and ethyl cresol. The remaining peaks were not identified but they were used to correct for background peaks when analyzing extracts of treated or spiked urine.

Results of the analysis of the spiked urine samples show that a significant quantity of the sulfide is recovered as sulfoxide, a significant quantity of the sulfoxide is recovered as sulfone, and essentially all of the sulfone is recovered as sulfone (Table 91). These data reflect the relative rate of _in vitro_ interconversion based on the assumption that the response factors by GC-MS are equal for all three compounds.

Results of analyses of the 24-hour urine specimens obtained from rats treated with either sulfide, sulfoxide, or sulfone are presented in Table 92. These data reflect the relative rate of _in vivo_ interconversion of the test compounds plus any in vitro interconversion that occurred during the collection process. Again the assumption has been made that response factors are equal for all three compounds. Urine from rats treated with sulfide was found to contain a 5:1 ratio of sulfone to sulfide with very little sulfoxide present. Urine from rats treated with sulfoxide was found to contain a 25:1 ratio of sulfone to sulfoxide with no sulfide detectable. Urine from rats treated with sulfone contained only sulfone.

It should be pointed out that approximately 60% of the total dose is excreted in the urine of rats within the first 24-hours based on the pharmacokenetics studies using radiolabeled compounds. Approximately 6% of this ^{14}C in the urine is extractable with chloroform prior to enzyme hydrolysis. This means that for each treatment the relative concentrations in Table 92 account for less than 4% of the administered dose.

Significant quantities of sulfide and sulfoxide undergo oxidation _in vitro_ in urine from untreated rats. However, the rates of oxidation are greatly enhanced _in vivo_ resulting in almost complete interconversion of sulfide and sulfoxide to sulfone. No evidence of sulfur reduction was found for sulfone or sulfoxide in either the _in vitro_ or _in vivo_ studies. These data account only for compounds in the free, chloroform-extractable form and not for conjugated material.

TABLE 91. RELATIVE CONCENTRATIONS OF SULFIDE, SULFOXIDE, AND SULFONE IN SPIKED URINE.

Sample	Relative Concentration[a]		
	Sulfide	Sulfoxide	Sulfone
Sulfide Spiked	1.00	0.85	0.02
Sulfoxide Spiked	0.01	1.00	0.51
Sulfone Spiked	0.01	0.01	1.00

(a) Normalized to major constituent.

TABLE 92. RELATIVE CONCENTRATION OF SULFIDE, SULFOXIDE, AND SULFONE IN URINE FROM TREATED RATS.

Sample	Relative Concentration[a]		
	Sulfide	Sulfoxide	Sulfone
Sulfide Treated	0.19	0.02	1.00
Sulfoxide Treated	0.01	0.04	1.00
Sulfone Treated	0.01	0.01	1.00

(a) Normalized to major constituent.

AMES BACTERIAL MUTAGENESIS BIOASSAY

METHODS AND MATERIALS

The Ames bacterial mutagenicity test is a bioassay designed to detect potential mutagens by means of a special set of five Salmonella typhimurium strains developed by Dr. Bruce Ames. Specifically these are TA-1535, TA-1537, TA-1538, TA-98, and TA-100. The assay is based on the property of these five strains to reversion from a histidine requiring state to prototrophy due to exposure to various classes of mutagens. The histidine deficient variant strains are used to detect frameshift mutations (TA-1537, 1538, and 98) or base pair substitutions (TA-1535 and 100). These tester strains were developed for their sensitivity and specificity to be reverted back to the wild type by particular mutagens.

The assay has been adapted for use in detecting compounds which may be potential mutagens. It has been documented that most compounds that act as carcinogens in mammals also act as mutagens in bacterial systems. However, a significant percentage of known carcinogenic compounds are not active carcinogens in the parent form, but require enzymatic alteration to an active moiety. Mammalian microsomal hydroxylase systems are responsible for this activation. Since these specific bacteria do not have the mammalian microsomal enzyme system, mammalian liver homogenates are added to the system to activate the nonmutagenic parental compounds to possible mutagens.

The materials and preliminary steps in the preparation of the liver microsomes and bacteriological plating media which are used in toxicological and mutagenicity studies of compounds using the Ames standard Salmonella tester strains are described below.

The activation system for mutagenesis screening in these studies consisted of Arochlor 1254 and phenobarbital induced microsomes derived from rat livers. Arochlor 1254 induction was accomplished by a single intraperitoneal injection (diluted to 200 mg/ml in corn oil) into adult male rats weighing about 200 g each, at a dosage of 0.5 mg/g of body weight,

5 days before sacrifice. The rats were deprived of food and water 24 hours before sacrifice. Phenobarbital induction was accomplished by the addition of sodium phenobarbital (0.1% concentration) to the drinking water for 5 days prior to sacrifice. The rats were stunned by a blow on the head and decapitated.

The livers were aseptically removed from the rats and placed into a cold preweighed beaker containing 10 ml of 0.15 M KCl. After the livers were swirled in this beaker, they were removed with forceps to a second beaker containing 3 ml of the KCl solution per gram of wet liver weight. The livers were then minced with sterile scissors, transferred to a chilled glass homogenizing tube and homogenized by passing a low-speed motor driven pestle through the livers a maximum of four times. The homogenates were then placed in cold centrifuge tubes and centrifuged for 10 minutes at 9000 G at 4 C. The resulting supernatant was decanted, aliquoted in 3 ml amounts to small culture tubes, quickly frozen in dry ice, and stored at -80 C in a Revco freezer. Sufficient microsomes for use each day were thawed at room temperature and kept on ice before and during use.

The S-9 microsomal mix was prepared according to the recommendations of Ames. The mix contains per ml: S-9 (0.15 ml); $MgCl_2$ (8 µmoles); KCl (33 µmoles); glucose-6-phosphate (5 µmoles); NADP (4 µmoles); and sodium phosphage, pH 7.4 (100 µmoles). Stock solutions of NADP (0.1 M) and glucose-6-phosphate were prepared with sterile water, aliquoted in appropriate amounts, and maintained in a Revco freezer. The stock salt solutions were prepared, autoclaved, and refrigerated. The S-9 mix was prepared fresh daily and was maintained on ice before and during use.

The Salmonella tester strains were obtained directly from Ames and stock solutions of the strains which had been stored in the Revco freezer. At monthly intervals, new bacterial isolates were obtained from this stock supply. Each clonal culture was checked for confirmation of biochemical activity and spontaneous reversion rate. The cultures which conformed to the specifications of Ames were streak isolated and used as master cultures. These master cultures were used as the origin

of weekly preparations of working broth cultures. All broth cultures were nutrient broth (Difco) supplemented with 0.5% NaCl. The bacterial cultures to be used for an assay were prepared by inoculating 0.1 ml of each strain into 10 ml of nutrient broth and incubating the culture in a water bath shaker for 16 hours.

The selective basal medium for all histidine requiring strains used in mutagenesis was 1.5% Bacto-Difco agar in Vogel-Bonner Medium E with 2% glucose. The basal medium used in the toxicity assays was the same medium fortified with 30 μg/ml of histidine.

The top agar (0.6% Difco agar, 0.5% NaCl) was prepared in 100 ml aliquots, autoclaved, and stored at room temperature. Before use in mutagenesis assays, the agar was melted and 10 ml of a sterile solution of 0.5 mM λ-histidine, HCl, and 0.5 mM biotin was added to the molten top agar and mixed thoroughly. For toxicity assays the molten agar was supplemented with 30 μg/ml of histidine. The top agar was then aliquoted in 2 ml amounts in sterile culture tubes and maintained at 45 C in a water bath before use.

The purpose of the positive control assay was to confirm the performance of the test components with and without microsomal activation and to provide a standard against which any activity of the test substances could be compared. The solvents for all stock control chemicals were a spectrophotometric grade dimethyl sulfoxide. The following compounds were used for positive control assays.

Indicator Strain	Nonactivation Assays	Activation Assays
TA-1535	sodium azide	2-aminoanthracene
TA-1537	quinacrine hydrochloride	2-aminoanthracene
TA-1538	2-nitrofluorene	2-aminofluorene
TA-98	2-nitrofluorene	2-aminofluorene
TA-100	2-nitrofluorene	2-aminofluorene

A complete test for each substance was comprised of

(1) A toxicity test

(2) A mutagenesis test

(3) A positive control test

(4) A sterility control test.

These tests were performed concurrently with a specific culture of a given bacterial strain and sample.

The toxicity determinations were made in triplicate for each compound against the selected tested strains. The toxicity of the sample materials was determined in both nonactivation and induced activation environments. The determinations were made as follows. The stock culture of each tester organism was diluted in physiological saline to yield approximately 100-300 colonies per plate when 0.1 ml aliquots were added to the molten agar overlay tubes and poured onto the minimal Vogel-Bonner glucose agar plates. Each of these plates was supplemented with 30 µg/ml of histidine to ensure that his-cells would form colonies when plated. A duplicate series of overlay tubes each with 300 cells were set up for both activated and nonactivated assays. Appropriate dilutions of each extract were added to the overlay tubes in 100 µl amounts. The activation system, when used, was added after both the tester strain and the test material had been added and just prior to the pouring. The overlay tubes were poured onto the histidine-supplemented glucose minimal agar plates. After solidification the plates were incubated at 37 C for 72 hours after which the resulting colonies were counted.

The plate test procedures for mutagenicity were conducted in a manner similar to the toxicity determination with the exceptions as noted below. A 0.1-ml aliquot of the broth culture was added to the 2 ml of molten top agar supplemented with a trace of histidine and biotin. For nonactivation tests, five dose levels of the test compound were added to the tubes and subsequently poured over the selective agar plates.

In activation tests, 0.4 ml of the appropriate S-9 mix was added to the top agar tubes after the test materials had been added and just prior to pouring. The poured top agar was allowed to solidify before

the plates were incubated. Following an incubation period of approximately 72 hours, the number of colonies growing on each plate was counted.

The Ames bacterial mutagenesis assay is extremely simple yet highly efficient in detecting mutagenic compounds. It has been shown to facilitate detection of nanogram quantities of some pure compounds.

The mechanism by which the mutagen is detected is by the reverse mutation of the cell to prototroph or the wild form. This wild form is no longer histidine dependent. However, each of the strains used has a constant rate of spontaneous reversion. Since the tester strains spontaneously revert, test materials which are only very slightly mutagenic must have a reversion rate sufficiently greater than the spontaneous rate in order to be detected. The toxicity results provide an accurate estimate of the toxicity of the material for the indicator cells under the specific treatment conditions. The bacterial colony counts of the replicate plates for each concentration of the test material and its corresponding control (spontaneous reversions) for each tester strain were averaged. The relative viability for each set of test conditions were calculated. The relative viability was described as the ratio of the average colony counts of the test plates to control plates. This ratio was used to express the toxic effect of the materials tested and represented the percentage of the inoculated cells which survived the toxic effects of the material. Thus, the control plates for each activated/nonactivated environment were assigned a value of 1.00.

The number of revertant colonies on the replicate mutagenesis plates were averaged for each concentration of test material and control. The average number of revertants on test plates was corrected for the toxicity of the concentration of the test material. This adjusted average number of revertants was calculated using the following formula:

$$\frac{1.00}{\text{relative viability}} \times \text{number of revertant colonies} \quad .$$

The adjusted average number of revertants was used to determine the relative mutagenicity for each test material and tester strain. This

index was calculated as the ratio of the adjusted number of revertants to the average number of spontaneous revertants on the control plates. This index was used to ascertain the mutagenicity of the sample assayed. An index value of 1.0 or less translates into no mutagenic activity. Thus the number of adjusted mutants for a particular dosage was equal in number to the number of spontaneous revertants of the tester strain when not exposed to a mutagenic environment. The greater the value of the number beyond 1.0, the greater the possibility of mutagenic activity. Index values of 2.0 to 3.0 and perhaps higher are best accountable to chance variation and human error although weak mutagenic activity cannot be overlooked. Values approaching 10.0 and higher are clearly indicative of activity.

The positive control assay was conducted with each test. These data are unrelated to either the toxicity or the mutagenic data except that it was obtained using the same tester strain population. The value of the positive control data was to confirm that the tester strains were capable of detecting the presence of substances which were mutagens in their own right as well as those substances which required activation for mutagenesis.

The negative or sterility control data were ascertained for the microsomal activation system, the solvent used for the materials used in the investigations, and the highest concentration of the test material. These negative test materials were incorporated into the histidine-supplemented top agar and poured over the surface of nutrient agar plates.

RESULTS

The results of the mutagenic bioassay analyses of the three related compounds using Arochlor 1254 as an inducer are shown in Tables 93 through 95, and using phenobarbital as an inducer are shown in Tables 96 through 98. The data presented in these tables show the average number of revertants obtained from the duplicate replications by tester strain and concentration of the compounds. The relative mutagenicity determined by

TABLE 93. MUTAGENICITY OF p-CHLOROPHENYL METHYL SULFIDE-AROCHLOR 1254 INDUCTION SERIES

Tester Strain	µl of Test Material	Activation		Nonactivation	
		Average Number of Revertants	Relative Mutagenicity	Average Number of Revertants	Relative Mutagenicity
TA-1535	100	Toxic		Toxic	
	10	3	0.1	3	0.1
	1	19	0.8	21	0.8
	0.1	16	0.7	12	0.4
	0.01	23	1.0	20	0.7
	0	23		28	
TA-1537	100	Toxic		Toxic	
	10	Toxic		Toxic	
	1	5	0.6	7	0.7
	0.1	9	1.1	10	1.0
	0.01	6	0.8	7	0.7
	0	8		10	
TA-1538	100	Toxic		Toxic	
	10	28	0.8	3	0.1
	1	29	0.8	7	0.2
	0.1	32	0.9	24	0.7
	0.01	33	0.9	27	0.8
	0	37		32	
TA-98	100	Toxic		Toxic	
	10	26	0.5	4	0.1
	1	39	0.8	31	0.8
	0.1	38	0.7	38	1.0
	0.01	45	0.9	34	0.9
	0	51		39	
TA-100	100	Toxic		Toxic	
	10	61	0.4	11	0.1
	1	143	0.7	150	0.9
	0.1	158	0.9	136	0.8
	0.01	158	0.9	150	0.9
	0	169		170	

TABLE 94. MUTAGENICITY OF p-CHLOROPHENYL METHYL SULFOXIDE-AROCHLOR 1254 INDUCTION SERIES

Tester Strain	µl of Test Material	Activation		Nonactivation	
		Average Number of Revertants	Relative Mutagenicity	Average Number of Revertants	Relative Mutagenicity
TA-1535	500	19	0.8	30	1.3
	100	22	0.9	22	0.9
	10	20	0.8	22	0.9
	1	22	0.9	28	1.2
	0.1	21	0.9	27	1.1
	0	24		24	
TA-1537	500	7	0.8	8	1.0
	100	9	1.0	6	0.8
	10	6	0.7	8	1.0
	1	8	0.9	7	0.9
	0.1	9	1.0	9	1.1
	0	9		8	
TA-1538	500	30	0.9	27	0.9
	100	29	0.9	27	0.9
	10	30	0.9	32	1.1
	1	28	0.9	29	1.0
	0.1	30	0.9	33	1.1
	0	32		30	
TA-98	500	35	0.9	29	0.5
	100	39	1.0	49	0.9
	10	38	1.0	36	0.7
	1	34	0.9	41	0.8
	0.1	36	0.9	39	0.7
	0	38		54	
TA-100	500	145	0.9	139	0.9
	100	137	1.0	144	0.9
	10	146	1.0	138	0.9
	1	134	0.9	124	0.8
	0.1	133	0.9	147	1.0
	0	144		155	

TABLE 95. MUTAGENICITY OF p-CHLOROPHENYL METHYL SULFONE-AROCHLOR 1254 INDUCTION SERIES

Tester Strain	Concentration µg/Plate	Activation		Nonactivation	
		Average Number of Revertants	Relative Mutagenicity	Average Number of Revertants	Relative Mutagenicity
TA-1535	500	20	1.1	24	1.0
	100	22	1.2	24	1.0
	10	22	1.2	20	0.9
	1	17	0.9	20	0.9
	0.1	20	1.1	23	1.0
	0	18		23	
TA-1537	500	5	0.7	5	0.7
	100	6	0.9	4	0.6
	10	8	1.1	4	0.6
	1	6	0.9	5	0.7
	0.1	6	0.9	5	0.7
	0	7		7	
TA-1538	500	27	0.8	24	0.9
	100	33	1.0	28	1.0
	10	28	0.9	29	1.0
	1	30	0.9	27	0.9
	0.01	25	0.8	24	0.9
	0	32		28	
TA-98	500	34	0.8	38	0.9
	100	32	0.8	31	0.8
	10	37	0.9	34	0.8
	1	40	1.0	38	0.9
	0.1	38	0.9	40	0.9
	0	42		41	
TA-100	500	129	0.8	148	1.0
	100	134	0.9	153	1.0
	10	140	0.9	132	0.9
	1	157	1.0	139	0.9
	0.1	149	0.9	138	0.9
	0	152		148	

TABLE 96. MUTAGENICITY OF p-CHLOROPHENYL METHYL SULFIDE-PHENOBARBITAL INDUCTION SERIES

Tester Strain	µl of Test Material	Activation		Nonactivation	
		Average Number of Revertants	Relative Mutagenicity	Average Number of Revertants	Relative Mutagenicity
TA-1535[a]	0.3	5	0.5	Toxic	NA
	0.1	14	1.4	16	0.9
	0.05	12	1.2	19	1.1
	0.03	5	0.5	8	0.4
	0.01	16	1.6	23	1.3
	0	10		18	
TA-1537[a]	0.3	4	0.3	Toxic	NA
	0.1	9	0.6	6	0.9
	0.05	11	0.7	11	1.6
	0.03	3	0.2	4	0.6
	0.01	10	0.7	8	1.1
	0	15		7	
TA-1538[a]	0.3	11	0.4	Toxic	NA
	0.1	34	1.4	15	1.2
	0.05	28	1.1	12	0.9
	0.03	10	0.4	8	0.6
	0.01	28	1.1	14	1.1
	0	25		13	
TA-98[a]	0.3	21	0.6	Toxic	NA
	0.1	30	0.8	17	0.8
	0.05	37	1.0	21	1.0
	0.03	13	0.4	8	0.4
	0.01	36	1.0	23	1.1
	0	37		21	
TA-100[a]	0.3	62	0.6	Toxic	NA
	0.1	95	0.9	83	0.8
	0.05	94	0.8	101	0.9
	0.03	73	0.7	91	0.8
	0.01	116	1.0	96	0.9
	0	111		108	

(a) Averages of three assays.

TABLE 97. MUTAGENICITY OF p-CHLOROPHENYL METHYL SULFONE-PHENOBARBITAL INDUCTION SERIES

Tester Strain	Concentration µg/Plate	Activation		Nonactivation	
		Average Number of Revertants	Relative Mutagenicity	Average Number of Revertants	Relative Mutagenicity
TA-1535[a]	1000	10	1.1	20	1.3
	300	9	1.0	20	1.3
	100	10	1.1	18	1.2
	30	11	1.2	15	1.0
	10	8	0.9	16	1.1
	0	9	1.0	15	
TA-1537[a]	1000	7	1.2	5	0.8
	300	7	1.2	5	0.8
	100	5	0.8	6	1.0
	30	7	1.2	6	1.0
	10	7	1.2	6	1.0
	0	6		6	
TA-1538[a]	1000	18	1.1	13	1.0
	300	17	1.0	12	0.9
	100	18	1.1	12	0.9
	10	20	1.2	16	1.2
	0	17		13	
TA-98[a]	1000	22	0.8	20	0.9
	300	30	1.1	22	1.0
	100	22	0.8	26	1.2
	30	24	0.9	21	1.0
	10	28	1.0	27	1.2
	0	27		22	
TA-100[a]	1000	96	0.9	107	0.9
	300	106	1.0	104	0.9
	100	111	1.0	94	0.8
	30	111	1.0	103	0.9
	10	112	1.0	107	0.9
	0	107		113	

(a) Average counts of two assays.

TABLE 98. MUTAGENICITY OF p-CHLOROPHENYL METHYL SULFOXIDE-PHENOBARBITAL INDUCTION SERIES

Tester Strain	µl of Test Material	Activation		Nonactivation	
		Average Number of Revertants	Relative Mutagenicity	Average Number of Revertants	Relative Mutagenicity
TA-1535	1000	12	1.2	32	1.2
	300	13	1.3	24	0.9
	100	5	0.5	26	1.0
	30	9	0.9	32	1.2
	10	12	1.2	27	1.0
	0	10		26	
TA-1537	1000	13	1.3	6	1.0
	300	10	1.0	6	1.0
	100	12	1.2	6	1.0
	30	10	1.0	5	0.8
	10	13	1.3	7	1.2
	0	10	1.0	6	
TA-1538	1000	19	0.8	15	1.1
	300	22	0.9	24	1.7
	100	25	1.0	8	0.6
	30	17	0.7	16	1.1
	10	23	0.9	12	0.9
	0	25		14	
TA-98	1000	27	1.0	22	1.2
	300	38	1.4	27	1.4
	100	30	1.1	32	1.7
	30	29	1.0	17	0.9
	10	23	0.8	19	1.0
	0	28		19	
TA-100[(a)]	1000	89	0.7	128	1.1
	300	105	0.9	115	1.0
	100	110	0.9	112	1.0
	30	115	0.9	118	1.0
	10	114	0.9	117	1.0
	0	123		117	

(a) Averages of two assays.

concentration of anthraquinone for each tester strain is likewise presented. This index is calculated as the ratio of the average number of revertants under test conditions to the average number of spontaneous revertants on the control plates. This index is used to ascertain the mutagenicity of the sample analyzed. An index value of 1.0 or less translates into no mutagenic activity. Thus, the number of adjusted mutants for a particular dosage is equal in number to the number of spontaneous revertants of the tester strain when not exposed to a mutagenic environment. The greater the value of the number beyond 1, the greater the probability of mutagenic activity. Index values of 2.0 to 3.0 are best accountable to chance variation and human error although weak mutagenic activity cannot be overlooked. Values approaching 10.0 and higher are clearly indicative of activity.

The data shown in Tables 14 through 19 show no suggestions of mutagenic activity associated with any of the tester strains. The sulfide was far more toxic to all tester strains than either of the other two samples. Aside from toxicity, there were no detectable differences between the responses elicited by the three chemicals on the tester strains. Metabolic activation did not potentiate the activity of any of the compounds.

The positive control data for the Arochlor 1254 and phenobarbital bioassay series are presented in Tables 99 and 100, respectively. These data are an averaged value for each of the six individual assays conducted. While each of the chemicals was examined several times in separate assays, the tester strains were derived from a single population. The value of the positive control data is to confirm that the tester strains are capable of detecting the presence of substances which are mutagens in the nonmetabolized form as well as the metabolized intermediates.

The negative or sterility control data are not included. The sample and test components were ascertained to be free of contamination. The microsomal activation mix was filter sterilized and was confirmed by culture.

The results indicate that at the test concentrations employed in this assay, none of the three chemicals is mutagenic either in the parent form or when activated by microsomal enzymes induced by Arochlor 1254 or

TABLE 99. POSITIVE CONTROL DATA - AROCHLOR 1254
INDUCTION SERIES

Compound	Concentration µg/Plate	Metabolic Activation	Histidine-Positive Revertants per Plate				
			1535	1537	TA-1538	98	100
Sodium azide	10	-	1393				
Quinacrine HCl	200	-		1395			
2-nitrofluorine	10	-			1405	1110	1086
2-aminoanthracene	10	+	432	298			
2-aminofluorine	10	+			2392	2252	1452

TABLE 100. POSITIVE CONTROL DATA - PHENOBARBITAL INDUCTION SERIES

Compound	Concentration µg/Plate	Metabolic Activation	Histidine-Positive Revertants per Plate				
			TA-				
			1535	1537	1538	98	100
Sodium azide	1	-	273				
	5	-					1038
Quinacrine HCl	200	-		1069			
2-nitrofluorine	5	-			417	300	141
2-aminoanthracene	5	+	70	45	406	467	535

phenobarbital. The p-chlorophenyl methyl sulfide was apparently more toxic in the phenobarbital assay than in the Arochlor 1254 assay. This difference in toxicity was found to be due to the instability of the sulfide when dissolved in DMSO. Storage of the compound in DMSO resulted in a rapid decrease in toxicity with time. In the phenobarbital assays, the DMSO solutions were incorporated into the assay within 3 hours. In the Arochlor 1254 assay, DMSO solutions were stored for longer periods of time.

HEXOBARBITAL SLEEP TIME STUDIES

In order to determine whether hepatic microsomal enzymes are induced by treatment of rats with the chemicals p-chlorophenyl methyl sulfide, p-chlorophenyl methyl sulfone, or p-chlorophenyl methyl sulfoxide, a hexobarbital sleep time experiment was performed. The rationale for this approach is that hexobarbital, being a short-acting barbiturate, elicits an easily measured parameter (sleep time) in rodents, the duration of which is dependent on the rate of disappearance of the drug from the animal's blood stream. For hexobarbital the drug is cleared by oxidative metabolism as it passes through the liver.

The microsomal enzymes, which are responsible for drug metabolism, are inducible following exposure of animals to a wide variety of chemicals. This results in higher enzymatic activity and more rapid clearance of hexobarbital following its administration. This is reflected by shortened sleep time as compared to control animals with noninduced enzymes.

METHODS

Male Fischer 344 rats weighing 150-180 grams were obtained from Charles River, Inc. The animals were observed through a 10-day quarantine in-house prior to starting chemical dosing. Each rat was eartagged, weighed, and housed individually. Five groups of 10 rats each were assigned by using randomization tables. The table below depicts the treatments for each group.

Group	Chemical	Dose, Vehicle, and Route of Administration
1	Sulfone	50 mg/kg, corn oil, PO
2	Sulfide	50 mg/kg, corn oil, PO
3	Sulfoxide	50 mg/kg, corn oil, PO
4	Corn oil	5 ml/kg, neat, PO
5	Phenobarbital	175 mg/kg, IP, in distilled water

The chemical treatment was given on three consecutive days. On the fourth day, following a 12-hour fast, the rats were weighed and were given an intraperitoneal injection of hexobarbital, 175 mg/kg. The rats were dosed with hexobarbital in a random order. Both injections and observations were made blindly by the investigator. Times were recorded at which (1) hexobarbital was injected, (2) righting reflex was lost, and (3) righting reflex was regained.

Calculations were made for time to loss of righting reflex and duration of loss of righting reflex (sleep time). Means and standard errors were calculated for each group, and Student's t-test was performed to test for statistically significant difference between the control group (#4) and any of the pretreatment groups.

The dose of phenobarbital used has been previously shown to cause significant decreases in hexobarbital sleep times through the mechanism of induction of microsomal enzymes. This group served as a positive control.

The doses of the three experimental chemicals to be used were determined from the toxicity data (body weights and gross observations) obtained in the 28-day oral dosing studies. The dose used was the same for all chemicals and was the highest dose that caused no more than a 10% body weight deficit compared to control animals and produced no overt toxic symptoms. CNS depression was an allowable toxicity as this appeared to be a reversible symptom following small doses of these chemicals to rats.

A mean sleep time for a test group which is found to be significantly shorter ($p < 0.05$) than control sleep times strongly suggests that microsomal enzymes are being induced. If sleep times for test groups are not different from controls, this may mean that the chemicals do not induce the enzymes. A prolongation of sleep times would suggest either hepatic damage from the chemical treatment or a potentiation of hexobarbital's action at the CNS level. <u>In vitro</u> studies would be required to definitively describe the action of these chemicals on the hepatic microsomes.

RESULTS

The dose of hexobarbital was increased from 75 mg/kg to 175 mg/kg after 10 animals did not lose their righting reflex following injection of the lower dose. Data for these animals were excluded as were data of two animals that did not sleep (improper injections) after treatment with 175 mg/kg of hexobarbital. Data from one animal were not recorded.

The experimental results are given in Table 101. The F-test yielded no differences in the group variances using the 99% level of significance. Group means were compared to control using the t-test. Significant differences ($p < 0.001$) were found in all treatment groups. These results provide evidence for an inductive effect on microsomal enzymes by the three test compounds.

Studying these enzymes by *in vitro* methods would provide a more specific examination of the hepatic enzymes involved in the metabolism of the test compounds.

TABLE 101. SLEEP TIMES OF FISCHER 344 RATS RECEIVING A 175 mg/kg IP INJECTION OF HEXOBARBITAL (MEAN VALUES AND STANDARD ERRORS)

Compound	Dose (mg/kg)	Number of Animals	Sleep Time (Minutes)
Control	0	6	35.7 (1.2)
Phenobarbital	75	9	18.8 (1.8)*
Sulfide	50	9	17.8 (1.8)*
Sulfoxide	50	6	18.7 (1.5)*
Sulfone	50	8	19.6 (1.4)*

*Significantly different from controls ($P<0.001$)

GENERAL DISCUSSION

Results of the LD_{50} determinations indicate that male rats were more resistant to these compounds than were females. The toxicity of sulfide and sulfoxide was similar while sulfone was slightly more toxic in rats. Mice were more sensitive than rats to sulfide and sulfone. Sulfoxide was more toxic than the other two compounds in both male and female mice.

Lethality could only be established in the acute dermal studies with sulfide. It was necessary to use a 24-hour exposure to gauze pads impregnated with large amounts of the test compounds in order to achieve lethality which only occurred in sulfide-treated rats exposed to 5630 mg/kg. Rats treated with sulfide and sulfoxide at that level experienced decreased motor activity, loss of consciousness, incoordination, dyspnea, diarrhea, and lacrimation with gradual recovery over a 5-day period. There was no evidence of abnormal clinical signs in animals treated with sulfone.

Skin and eye irritation studies were consistent with regard to responses of individual compounds. Sulfide did not produce a positive response in either study; sulfone produced mild responses while more intense reactions were illicited by sulfoxide, especially in the eye irritation studies. The only responses to skin sensitization studies occurred with sulfone and sulfoxide and those responses were equivocal.

Food consumption for rats in the 91-day study was similar for all compounds. Decreased food consumption was profound during the first 4 weeks and at higher dosage levels of sulfide during the first 8 weeks. By the end of the study, food consumption was generally at or higher than control levels except at the highest dosage level of sulfide. Food consumption and feed efficiency was extremely variable for treated mice but was generally decreased below control levels. Body weight gains were decreased in rats for nearly all dosage groups in all compounds. Severity of this change was consistent with the dosage level. The early decrease in food consumption with later return to or above control levels was apparently, at least partially, the result of the low palatability of sulfone-, sulfide-, and sulfoxide-containing feed. However, toxicity and not

palatability alone was clearly a factor in the decreased body weight gains which were substantially greater than the decreased food consumption. This was clearly demonstrated in mice wherein the greatest decrease in weight gains occurred, in some instances, during the initial 30-day period, a time during which food consumption was near control levels.

The most profound clinical manifestations of toxicity included central nervous system depression in rodents in the highest dosage groups from the acute oral (LD_{50}) studies and 28-day gavage studies. This effect was evident only in animals from those studies while emaciation, diarrhea, depressed weight gain, and ocular and nasal discharges were evident in animals from higher dosage levels of the 91-day studies. The lack of CNS effects in dosed feed studies may have been due to the palatability of the dosed feed which limited intake at the higher levels. Clinical signs in monkeys included anorexia, depression, diarrhea, and emesis. The depression observed in monkeys at the higher dosage levels included general lassitude and loss of responsiveness which may have been a similar effect to that observed in rodents in the high dose gavage administrations in the LD_{50} and 28-day studies.

Histologic manifestations of toxicity were substantially different in rodents as compared to monkeys. Predominant lesions in rodents were limited to hepatic megalocytosis with syncitial cell formation and necrosis together with flattening and loss of bronchiolar epithelium in mice at the highest dosage levels of all three groups, being most prominent in sulfone-treated animals. The substantially increased liver weights observed in rats and mice correlate with the pathologic changes observed in the liver and with the microsomal enzyme induction indicated by hexabarbitol sleep time studies.

Increases in kidney weights occurred in rats and mice, especially in lower dosage groups and in monkeys at high dosage levels. There were no histologic lesions observed in the kidneys of mice or rats; however, only the highest dosage groups were examined histologically. Renal lesions were observed in monkeys; however, the nature of the lesions and the numbers of animals involved are not adequate to explain the increased renal weights.

Hepatic lesions were also prominent in monkeys and consisted primarily of hepatocyte vacuolation, degeneration, and necrosis with megalocytosis evident in only one animal. These changes were most profound in sulfide-treated animals and probably correlate with the increase in liver weights observed in the higher dosage groups. Significant histologic lesions occurred in the lymphoreticular system of several monkeys representing all three compounds and all dosage groups. These lesions, which consisted of diffuse or nodular proliferations of large lymphoreticular cells involving lymph nodes, spleen, and bone marrow. These lesions were present in animals that had died or were terminated in moribund condition as early as 9 days after the initial treatments. Obviously, the lesions were at an early stage of development. These changes were considered to be reactive except in the case of the single nodular lesion in the mesenteric lymph node of the animal given 5 mg/kg of sulfone which was considered to be neoplastic. Other lesions such as thymic atrophy and depletion, generalized lymphoid depletion, and lymphoreticular hyperplasia were further evidence of the effects of these compounds on lymphoid elements in rhesus monkeys. It was interesting that no lymphoid involvement was observed in rodents in the 91-day study.

The only other lesions which were consistently present in rhesus monkeys, especially at higher levels, were vacuolization and/or degeneration of gastric mucosa and deep crypt areas of the small intestine and hyperplasia with or without vacuolization and degeneration in the adrenal cortices. The adrenal lesions correlate well with increased adrenal weights recorded at the higher levels. Lesions in the kidneys and thyroid glands occurred with less frequency.

The lesions which occurred in the bronchiolar epithelium in lungs of mice treated with all three compounds may have resulted from inhalation of the compounds. Mice often sleep in their feed cups a substantial amount of the time. Since these compounds are somewhat volatile, it is entirely feasible that there was adequate exposure to these compounds by the

inhalation route to induce these lesions. These lesions were consistently present in all mice given 3000 ppm of sulfone while only 15% and 16% of the mice given sulfide and sulfoxide were affected. Again, this may relate to the relative volatility of these compounds.

Changes in several hematologic or clinical chemistry parameters were comparable for rats and monkeys. Mild decreases in hematocrit and hemoglobin values were generally observed in both rats and monkeys with inconsistent changes observed in monkeys given sulfone. Increases in BUN levels were prominent in most monkeys at high dose levels of all three compounds. BUN values were significantly elevated in male rats which received sulfone. Although changes were observed microscopically in the kidneys of several monkeys, they were generally not of such severity as would be necessary to induce an increase in the BUN level. The reason for the rise in BUN levels in rats and monkeys was not apparent. Alkaline phosphatase levels were mildly depressed in both rats and monkeys. Although food consumption may be positively correlated with alkaline phosphatase in adult rats, this factor was probably not responsible for the observed decreases since food consumption of rats in the 750 ppm groups was higher than control food consumption. Cachexia and malnutrition may also cause decreased alkaline phosphatase levels; however, this too was not likely to have caused the changes in this study since both rats and monkeys at lower dosage levels were not cachectic. Biliary obstructions in the rat may produce a decrease in serum alkaline phosphatase levels; however, a concomitant rise in SGOT levels would be expected. The direct interference of the test compounds with the determination for alkaline phosphatase cannot be ruled out.

The SGOT levels were mildly decreased in some groups of rats while increased SGOT levels were observed in some monkeys at higher dosage levels. The decreased levels of this enzyme in rats is difficult to explain on a physiologic basis; however, again the possibility that the test compounds may have interfered with the analytical procedures should be considered. Increased SGOT levels in monkeys may have been due to the hepatic lesions which were prominent in many high dose animals, especially those treated

with sulfide and sulfoxide. Concomitant increases in SGPT values in these animals were also consistent with hepatic parenchymal damage. The alteration of serum electrolytes in rats was profound. The increased potassium and decreased sodium levels are suggestive of adrenal cortical hypofunction. There were no corresponding changes in the adrenal cortical morphology; however, the rapid recovery suggests that this may have resulted from altered steroid levels without adrenal cortical degeneration or necrosis. The increase in calcium levels was also not substantiated by morphologic lesions. However, such factors as parathormone and vitamin D levels may have been involved and these parameters were not assessed.

Although the three compounds evaluated in these studies responded similarly in many respects, there were qualitative and quantitative differences in toxicity among the three chemicals. Analytical studies indicated that there was extensive in vivo conversion of sulfide and sulfoxide to sulfone. The variations which occurred in the toxic responses to these compounds indicate that these conversions were probably incomplete.

There was a profound increase in weight gains primarily in higher dosage groups during the recovery period in both rats and mice. Hematology and clinical chemistry values for both rats and monkeys following the 14-day recovery period were generally closer to or at control levels. In many instances, however, return to control levels was incomplete following the recovery period. The increased potassium and calcium levels observed in rats did, however, return to control levels. Likewise, the organ weights tended toward recovery to control levels. The increased renal and hepatic weights remained elevated but the increase was less than that which occurred at 13 weeks. Hepatic lesions observed histologically in mice were substantially less severe in animals terminated following the recovery period. Compound-induced lesions in monkeys were infrequent and mild in the recovery animals as compared to those terminated immediately following exposure.

A "no effect" level was not reached in either the rodent or monkey studies. Compound-induced changes in hematology and clinical chemistry parameters were present at all levels and pathologic changes, although decreased in severity, were still present. Likewise, body weight gains

were mildly to moderately decreased at the lowest levels in rodents. Pathologic changes were observed in monkeys treated at the lowest dosage levels although they were, for the most part, minimal. The exception was the lymphoreticular proliferative lesion observed in one animal treated with 5 mg/kg of sulfoxide. Mild clinical abnormalities and equivocal changes in hematology and clinical chemistry parameters also occurred in animals at the lowest levels.

CONCLUSIONS

- There was no evidence of mutagenicity for any of the three compounds in this study in the Ames bacterial mutagenesis assay.
- There was substantial in vivo conversion of sulfoxide and sulfide to sulfone in rats in this study.
- All three compounds are potent microsomal enzyme inducers as determined by hexobarbital sleep time studies.
- Major morphologic lesions in rodents occurred in the liver and lung.
- Major lesions in monkeys occurred in the lymphoid system, liver, gastrointestinal tract, kidney, and adrenal glands.
- All three compounds are capable of inducing lymphoproliferative lesions in rhesus monkeys after short exposure periods and at low dosage levels.
- The "no effect" level for all three compounds in $B_6C_3F_1$ mice and Fischer 344 rats is less than 750 ppm for 91-day studies.
- The "no effect" level for these compounds in rhesus monkeys is below 5 mg/kg.

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